DISSERTATION

THE USE OF ESTRUS SYNCHRONIZATION AND TRANSCERVICAL ARTIFICIAL INSEMINATION FOR OUT OF SEASON MANAGEMENT OF NAJDI SHEEP IN SAUDI ARABIA

SUBMITTED BY

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KHALID A. AL-SOBAYIL ENTITLED "THE USE OF ESTRUS SYNCHRONIZATION AND TRANSCERVICAL ARTIFICIAL INSEMINATION FOR OUT OF SEASON MANAGEMENT OF NAJDI SHEEP IN SAUDI ARABIA" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

COMMITTEE ON GRADUATE WORK

Advisor

Department Head

ABSTRACT OF DISSERTATION

THE USE OF ESTRUS SYNCHRONIZATION AND TRANSCERVICAL ARTIFICIAL INSEMINATION FOR OUT OF SEASON MANAGEMENT OF NAJDI SHEEP IN SAUDI ARABIA

The objective of this research was to evaluate the reproductive performance in both Rams and ewes of Najdi sheep during the time of the year when they exhibit low reproductive performance, April - September. In this study, the use of some reproductive management, such as: 1- Ram breeding soundness examination, 2- Estrus synchronization, 3- Transcervical AI and, 4- Pregnancy diagnosis by Real-time ultrasonic technique, were applied to Najdi sheep.

A breeding soundness examination (BSE) involving physical as well as reproductive tract examinations, scrotal circumference (SC), and semen evaluation was done on 82 Najdi rams at the sheep unit that belong to National Agriculture Development Company (NADEC), located approximately six hundred km north-west of Riyadh, the capital city of Saudi Arabia. The farm is about 799 m above the sea level and the average maximum temperature during the hot months is approximately 40 ^{o} C. Seventeen rams were classified as unsatisfactory breeders due to physical (10 rams) and reproductive (7 rams) problems. Semen evaluation showed that the majority of the tested Najdi rams had the ability to produce semen with adequate quality at the commencement of the hot months. The measurement of scrotal circumference showed that rams of this breed had smaller scrotal measurement ($\bar{X}$= 31cm), as compared to North American breeds that have in average 34 cm. This could be due to the relatively small size of this

breed and because the measurement took place at the time of low breeding performance. Twenty-two of the tested fertile rams were used for subsequent natural and artificial breeding.

The use of estrus synchronization and transcervical AI were evaluated on 300 Najdi ewes that were assigned equally into three groups: 1) Group 1: control ; no hormonal treatment and ewes of this group were exposed to 8 BSE tested fertile Najdi rams for 17 days; 2) Group 2: treatment 1; ewes were synchronized with control intravaginal drug releasing devices (CIDRs) that contain 3g progesterone for 12 days and at the time of CIDRs removal, the ewes injected with 600iu PMSG and bred naturally by 10 tested fertile rams that were introduced for seven days; 3) Group 3: treatment 2; ewes were treated the same as treatment 1 one in regard to hormonal treatments, CIDRs and PMSG, but the ewes were transcervically inseminated 48-52 hours after CIDRs removal with 100×10^6 sperm as fresh extended semen. The results showed that the treated groups had a significant ($\alpha < .01$) difference in regard to the number of ewes lambed and the number of lambs born per ewe. In addition, ewes in treatment 1 and 2 had shorter lambing period in comparison with the control group 9,10,21 days respectively.

The ewes in the first and second treatment that had essentially known breeding dates were examined by real-time ultrasonic technique. An external 5MHz probe was used in this study. The ewes were tested at three different stages of pregnancy, 20, 40, and 60 days post breeding. The results showed a significant ($\alpha < 0.01$) difference in the accuracy of pregnancy diagnosis in the three different diagnosing dates. The accuracy of pregnancy diagnosis was 66, 90, and 100% at 20, 40, and 60 days post breeding.

The economic aspect of using estrus synchronization, TCAI and real-time ultrasonography techniques were evaluated. The use of this reproductive technique decreased the cost of rams per ewe as well as the cost of ram per born lamb. This study started on April 22 and end on October 15, 1999.

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CHAPTER 1

NAJDI RAMS EXAMINATION

INTRODUCTION

Sheep have an important place in the nutrition for people in Saudi Arabia. The majority of the Saudians eat lamb as their first choice of red meat. The number of sheep has steadily increased throughout the last ten years. In 1990, there were approximately 7.2 million head of sheep and the number increased to 10.3 million in 1999 (1). The government of Saudi Arabia supports the industry of sheep by encouraging the establishment of new sheep enterprises. The agricultural department provide grants to anyone who wants to set up a new sheep project. In addition, the government imports grain such as corn and barley then sell them at reasonable prices to producers.

In the Kingdom of Saudi Arabia(KSA), there are many local breeds of fat-tailed sheep. The great majority of the sheep population in Saudi Arabia are Najdi Sheep (Figure 1). Najdi sheep are raised in the middle part of Saudi Arabia in an area called Najd from where the breed took its name. Najdi sheep are a hardy breed and survive well under poor feeding and extreme climatic conditions. They usually have a black body with white head, neck and legs, but it is not uncommon to see all white or black and white mixed animals. This breed characteristically has a very fat-tail (Figure 2) which could affect the success of natural mating.

Sheep in Saudi Arabia are raised either in small numbers by individuals on small farms or in huge numbers at large facilities. Recently, several sheep farms have been established that raised thousands of local sheep. One of these farms is National

Agriculture Development Company (NADEC) sheep project where approximately 120 thousand of sheep are raised in four different locations (2).

Figure 1: Najdi Sheep

Figure 2: The Fat-tail of Najdi Sheep

In general, the local sheep are less productive than those in temperate parts of the globe. They have no or very low reproductive performance at the end of spring and during the summer. In addition to low fertility, the sheep are smaller than sheep breeds from temperate climates and have yield lower quantities of meat. These facts affect the sheep industry. There are two ways to improve the sheep industry in KSA: introducing high performance sheep into the KSA, however, this is usually not successful due to the local environment. The second choice is to try to improve the performance of the local Najdi sheep by using new reproductive management techniques to increase their productivity .

In this study, some reproductive management techniques were used to increase the lambing rate of Najdi sheep. There were three major goals of this study:

1. Examine the potential fertility of Najdi rams at the end of their normal reproductive season and only using those with high potential fertility.
2. Synchronize Najdi ewes and artificially inseminate them by using trans- cervical techniques.
3. Real-time ultrasonic pregnancy diagnosis at different states of gestation.

This study was done at the sheep unit belonging to NADEC, which is one of the largest agricultural companies in Saudi Arabia. It was established in 1981 with capital of $106 million. Currently, this company has about 120,000 Najdi (65%) and Naemi (35%) sheep. The sheep unit was established in 1984 with only 5,406 sheep of these two breeds. The sheep numbers have increased throughout the last 16 years (Table 1).

Table 1 : Sheep numbers at NADEC farm

Year	Sheep #
84	5,406
87	16,242
90	37,269
93	74,481
96	95,200
99	116,000

The aim of raising sheep is to provide fresh lamb to consumers. The company has integrated slaughter, and storages facilities for their lambs as well as their own marketing techniques. The lambs are sold at 40 kg weight and this is when they are approximately 6 months of age (Table 2).

Table2: Average production performance of Najdi at the NADEC sheep unit.

Characteristics	Performance
Average body weight (kg):	
mature ram	65-75
mature ewe	50-60
Average birth weight (kg)	3-5
Average lambing rate (%)	60
twins(%)	15
Average weaning weight (45 days) (kg)	15-18
Shorn Fleece weight (kg)	1-2
Average weight at 6 months (kg)	
ram lambs (kg)	40-45
ewe lambs (kg)	35-40

Ram Fertility:

Increasing the number of offspring in flocks depends to a large degree on using males capable of producing high amounts of high quality semen. In natural selection, if the male has low fertility and is affected by other dominant males, he will sire fewer offspring. With domestication, man has eliminated males that produced reduced numbers or no offspring. Unfortunately, there is not an examination that gives an absolutely accurate indication of the ram's fertility and in multiple sire groups parentage is difficult to establish. However, through a Breeding Soundness Examination (BSE) prior to the breeding season, one can distinguish between rams with potentially satisfactory and unsatisfactory fertility (3).

Breeding Soundness Examination (BSE):

A BSE exams a ramines physical and reproductive capabilities and includes a semen test. The goal of BSE is to classify the potential breeding ability of each ram into one of four categories: 1) unsatisfactory breeder; 2) questionable breeder; 3) satisfactory breeder or 4) excellent breeder (3). A BSE is a quick and economic procedure which is recommend for examining males 30-45 days prior to use.

The breeding soundness examination was originally developed for cattle in Colorado in 1954 by H.J. Hill, L.C.Faulkner and L.Ball (5). The exam was developed following very cold winters in the late 40's where the fertility of range cattle was negatively affected due to frost bite of the bulls' scrotums (4,5). These evaluations were made possible by development of the bovine electro ejaculator (EE) to obtain semen samples from the bulls. The result of applying BSE to bulls in Colorado showed that more than 20% of the bulls that were tested classified either unsatisfactory or questionable potential breeders (5). For sheep, ram examinations have been fully established in the United Kingdom for more than 26 years. (6). The first effort to standardize the BSE for rams in the U.S.A., using an electroejaculator, was at the meeting of Western Regional Coordination Committee-46 in 1986. The standard protocol for ram BSE was established in 1987 (7).

Before the establishment of the current BSE protocol, Hulet et al. had done a series of trials on ram fertility at the U.S. Sheep Experiment Station (7,8,9,10). They reported a relationship between the semen quality of rams and the lambing rate. In addition, they determined that there is a high correlation between fertility and sperm

motility, semen pH and the percentage of abnormal sperm in a sample. There is no published data in applying BSE in any of the Saudian rams.

This complete test includes the examination of all the different aspects of a ram's reproductive function to estimate his potential breeding ability. Breeding soundness examinations can be broken down into several parts, which ideally include:

1. Physical and health examination
2. Reproductive organs examination
3. Semen evaluation
4. Libido testing

Physical Examinations:

A physical examination involves a thorough observation of all conditions, which may prevent or reduce the ram's breeding ability (11). The following must be examined: body condition, feet and legs, gait, eyes, teeth, mucous membranes, body temperature and the overall general health status of the ram (12). This examination will help to eliminate rams with either congenital or post-natal abnormalities. In addition, any health problems should be recorded. Some problems may not be detected by the owner, therefore, regular examinations are very important (12).

It is wise to maintain a ram in good body condition all year round, especially if the ram will be used to breed ewes during the entire year or if semen is to be collected from him during the entire year. This is very important because the sperm evaluated today would be affected by his health status two months ago. This is true because sperm production, maturation and the fertilization ability of the sperm takes approximately two

months (13). Body condition score (BCS) is a subjective evaluation with scores from one to five. A score of one is given to an emaciated animal while a score of 5 is given to an extremely fat animal. Some researchers recommend the use of half scores between 2 and 3 and between 3 and 4 (14). A BCS of 2.5 to 3.5 is recommended for rams prior to entering the breeding season (15).

Good feet and legs are essential for rams to service ewes and especially range rams that are expected to travel long distances . A ram must have a normal gait and should not be lame, either through limb or joint abnormalities or feet infections. There are many causes of lameness in sheep (16). Some examples are structural faults, such as sickle hocks and curved legs or infectious diseases like foot rot, scald, and sole abscesses. Lameness not only reduces the ability of the ram to successfully breed ewes, but infections may affect the active daily production of sperm (17).

Eyes should be clear and free of injuries or diseases such as persistent ocular discharge, cataracts, entropion and pink eye. Eye problems can reduce the ram's breeding effectiveness or allow him to be eliminated by other rams. There are some other general examinations that should be done, such as aging of the ram, teeth, wool, skin, head and body conformation.

Examination of the Reproductive organs:

A complete examination of the reproductive tract for disease and abnormalities should be made. The scrotum, testes, epididymis, penis and prepuce should be carefully examined. The scrotum contains the testes and the epididymis and plays a major role in protection of the testes and thermoregulation for sperm production. There should be no

sign of injury of disease such as dermatitis which can cause pain and may affect the movement of the animal. In addition, the scrotal circumference (SC) can be measured using a scrotal tape. This measurement is relatively related to many fertility parameters in cattle (3). It is also inversely related to the onset of puberty in both males and females (18). In rams, SC measurement is a good indicator of potential sperm production (19). For this reason, the Western Regional Coordination Committee on Ram Epididymitis and Fertility recommends that ram lambs and yearling rams should have SC of greater than 30and 33 cm, respectively (15). However many factors, such as age, breed, nutritional status and seasonality, can affect SC (15)(17).

The testes are responsible for production of sperm and male hormones. The testes should be palpated for size, shape, consistency and adhesions to the scrotum. Any unusual softness, firmness, swelling, atrophy or lack of tone or symmetry should noted. Consideration should be given to any aspect that may affect proper thermoregulation of the testes.

The epididymis can be divided into the head, body and tail. Sperm undergoes maturational changes while passing through the head and body of the epididymis. Sperm are stored in the tail of the epididymis and can retain fertilizing capacity for several weeks until breeding or lost with the urine (20). For this reason, the tail of the epididymis should be examined carefully. It should be large, rounded, resilient and should not have hard lumpy deformities (17). There are some infectious agents resulting in epididymitis, mature rams can be affected by *Brucella ovis* and young rams by *Actinobacillus spp* and *Histophilus spp* (15, 21). Infected rams may have a swollen

epididymis and abnormal sperm or leukocytes (white blood cells) in the ejaculate. Epididymitis causes significant economic loses to breeders.

Brucella ovis infection in sheep is the cause of a chronic epididymitis in rams that can result in infertility of sub fertility. The first reported infection in sheep was in New Zealand and in Australia 50 years ago (19). Infected rams may spread infection throughout the flock. Maintaining an infected ram will increase the cost of the sheep production (22). In addition, Brucellosis is a zoonosis, acquired from handling of infected animals. Annual serologic Enzyme Linked Immunosorbant Assay (ELISA) testing is recommended and infected rams should be culled.

The penis and prepuce can be examined during semen collection. The glans should be observed for abnormal shape or size. The presence or absence of the appendage, urethral process, should be noted. Any abnormality or infection of the penis should be observed. Palpation of the penis posterior to the scrotum will allow detection of injury. Finally the prepuce should be closely examined looking for signs of injury or abnormal discharge.

Semen collection and evaluation:

Semen evaluation will be done only when a ram has passed the physical and reproductive examination . Semen is collected from rams by either artificial vagina (AV) or with an electro ejaculator (EE).

The AV is constructed to be similar to an actual vagina. The semen is collected by having the ram mount an estrus ewe or trained to mount an other animal or an object. When the ram mounts, his penis is directed into the AV by the person collecting the

semen. Collection of semen by AV is explained in detail in Salmon's AI book (23). Libido and mating ability can be tested if this method is used.

Use of EE was first described by Genn in 1936 (5). This method can be used for rams that are not easily trained to use the AV. By using EE, libido can not be evaluated. The result is often a greater volume of semen but lower concentration of spermatozoa (23). This technique of semen collection is also explained in details in Salmon's AI book (23). It may be assumed that using EE is painful to the ram. However, using EE was not deemed to be more aversive than shearing (24).

Semen is immediately examined for volume, color, motility, morphology, concentration and presence of inflammatory or foreign cells. Most of these factors can be affected by age, season, temperature, breed and the collection method. Generally, semen from rams has low volume and high concentration. Normal values for mature ram semen are shown in table 3 (20).

Table 3: Normal seminal characteristics of ram semen

Semen characteristics	Value
Ejaculate volume (ml)	0.8-1.2
Sperm concentration (million/ml)	2000-3000
Sperm / ejaculate (billion)	1.6-3.6
Motile sperm (%)	60-80
Morphologically normal sperm (%)	80-95

The volume can be measured in milliliters directly from the graduated collection tube. Volume is affected by many factors such as the collection methods, sexual excitement before collection, age of the ram, breed and frequency of collection.

The color of semen depends on the number of sperm in the ejaculate. A creamy sample has very high sperm concentration where a watery sample has few sperm. Collected samples should be free of contaminates such as blood, dirt, urine or pus.

Motility can be defined as the percent of moving cells. Motility is essential for fertility but not necessarily an indication of fertilizing capacity because normal spermatozoa lose their fertilizing ability before loosing their motility (20). Sperm motility as well as muscular contraction and ciliary motion of the female reproductive tract are very important for the transportation of sperm from the site of deposition to the site of fertilization(18). It is necessary to use a microscope that has a warm stage to prevent cold shock. Sperm motility can be inhibited by contamination of sperm sample due to improper handling and also to some sperm cell defects (15).

Morphology examination is conducted to determine the percentage of abnormal spermatozoa in the ejaculate. Semen samples from most males contain some abnormally formed spermatozoa (20). However, if the percentage of abnormal sperm cells are higher than specific percentage, depending on species, the fertility can be affected. Rams that have higher than 30% abnormal cells should not be used for breeding as semen morphology is correlated with fertility and fecundity (7.8,9,10).

Understanding the physiology of sperm production and spermatic cycle is very important to eliminate those factors which increase the accident of having either primary or secondary abnormalities ((25). Spermatozoa are often examined under oil emersion

using a phase contrast microscope at 1000 x magnification using the morphology test as explained (15, 23).

The presence of white blood cells in the ejaculate should be looked for. They appear three times larger than a sperm's head and they look round and shiny or as refractory objects (15). Under high magnification, the type of WBC can be determined (15). The presence of WBC is an indicator of inflammatory lesions of the genitalia and its accessory glands. However samples that are contaminated by preputial fluid may contain an occasional WBC (15). The presence of large numbers of neutrophils is likely to reduce fertility (26) there should not be more than five WBC in a high power field (HPF) (12). In addition, cells other than WBC that may be seen in the semen sample. Some examples of these cells are: epithelial cells, erythrocytes and developing sperm cells. The presence of these cells should be observed and the reason behind their presence should be recognized and eliminated.

SPERM PRODUCTION IN RAMS:

Rams reach maturity at about 5-8 months of age. The sperm production and sexual activity of a young ram is driven by an increase of lutinizing hormone (LH) secretion during the pre-puberal period. This increase is due to the increase of gonadotrophin releasing hormone (GnRH) secretion and the change in negative feed back of testosterone on the GnRH-LH axises (27). Increasing LH secretion stimulates the Leydig cells in the testes to increase secretion of testosterone which is responsible for sexual activity. A ram with high serving capacity has more lambs per ewe , a shorter lambing season and had more uniform lamb crop (28). Rams are different in serving capacity and it has been estimated that around 10 to 20% of rams are either inactive or

have homosexual behavior (28). Libido is affected by several factors such as seasonality, genetics. rearing, environmental and even by the uterine environment surrounding the ram before his birth (28).

In the tropics where day and night are approximately equal lengths, the native sheep do not show seasonality in their breeding. This is not the case in northern or southern regions of the world. In these regions, sheep show a seasonal breeding period. Rams, as well as ewes, are affected by the change of daylight. The evidence of season effect on ram reproduction can be observed from the change of its reproductive activity, testes size, semen quality and follicle stimulating hormone (FSH) and LH concentration. When the day length starts to decrease, the amount of melatonin secretion form the pineal gland will increase. This increase will affect the hypothalamus which stimulates the secretion of FSH and LH form the anterior pituitary. FSH stimulates the production of sperm and the hormone inhibin from the testes. LH affects Leydig cells to increase its production of testosterone. In France, a study with Ile-de-france rams has shown a change in testes weight from the breeding season (300-320g) to the non-breeding season (180-190g). In addition, semen quality and fertility were affected by the change of the seasons (19). In another study, it was found that LH pulses increased from 3 pulses/24 hours during the non breeding season to 6 pluses/24 hours during the breeding season (19). In addition to day length, there are some other factors that affect the onset of breeding season. These include : weather, body condition and health. High summer temperature is often responsible for temporary infertility (20).

Advantages of BSE:

An annual BSE prior to the use of the ram should be carried out because it provides many advantages to the breeder. Some of these advantages are:

1.Using BSE can increase the profit of raising sheep by 10-12 dollars per ewes (11). In cattle, it has been estimated that a 6% increase in the calf crop weaned due to BSE would represent and approximate return of $17 for each $1 invested in BSE (3).

2. Improve male: female ratios. A mature ram with high fertility and sexual activity can cover more ewes in a short period.

3. Increase the fertility of the flock by increasing lamb output by 2-3% (6).

4. Prevent the spread of diseases which can transfer from an infected ram to the herd.

5. Using a high performance ram will insure breeding the ewes early so this will improve weaning weight.

Breeding soundness Examination of Najdi rams:

Introduction:

The productivity level of Najdi sheep is low partly because they reproduce only once a year. Production is also limited by low ewe fertility and low twinning rates. One reason for low productivity, could be the influence of ram on reproduction. Ram fertility especially at the time of low reproductive activity could affect conception rate. Information on the reproductive traits of Najdi rams is limited. Therefore, this study was conducted at the end of the active breeding season , that was in late spring when Najdi sheep have low breeding activity. The first objective of this study was to evaluate

information of breeding soundness examination for Najdi rams. The second objective was to select rams for use in subsequent natural breeding and artificial insemination studies.

Materials and Methods:

Eighty two mature Najdi rams were phenotypically selected from approximately 600 rams. The selected rams were evaluated for physical and health examination, reproductive tract examination and semen evaluation.

The 82 Najdi rams were housed at NADEC sheep unit located at 799 meters above sea level (27.29 N and 42.35 E). The annual rainfall of the area averages between 120 mm and the average minimum and maximum temperature are 46F (January) and 112F (July) respectively. A BSE was conducted in the third week of April 1999. Each ram was examined with emphasis on the general health and physical soundness, integrity of its genital tract and semen evaluation.

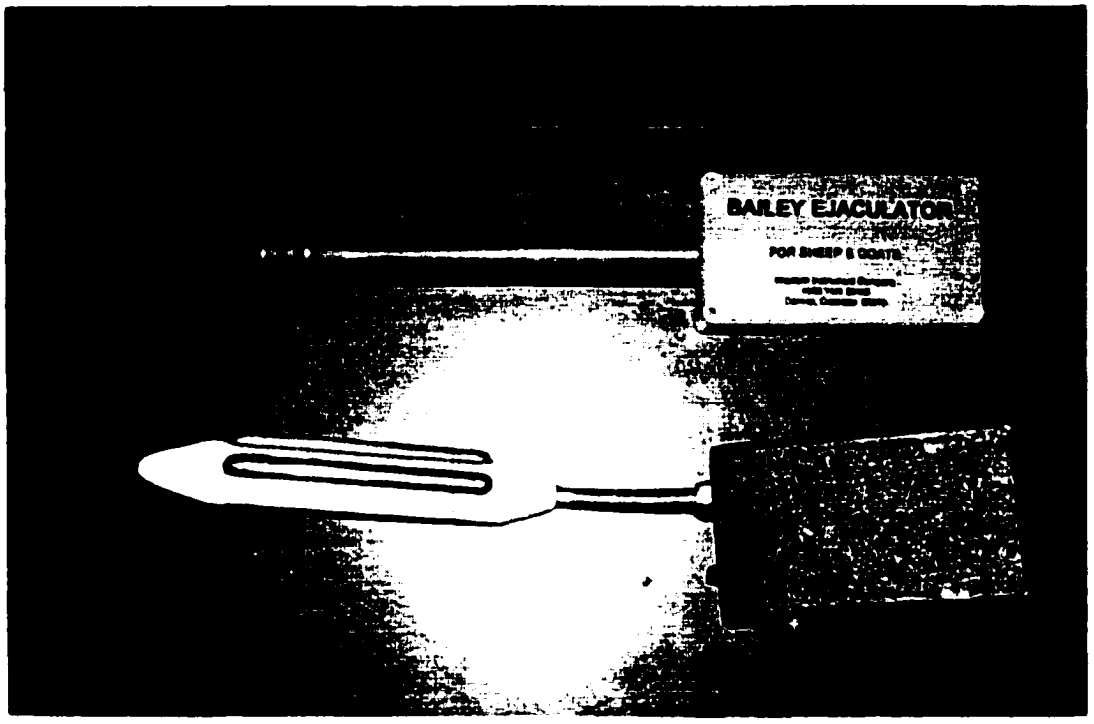
Data were collected in body condition, feet, legs, gait, eyes, skin and general health. Rams were restrained in a standing position and a subjective body condition score was given. Body condition scoring was done by using the hand to feel muscles and fat over the vertebrae in the loin region and also by the size of the fat-tail. Any observation of lameness was recorded. The condition of the mouth and teeth were observed and the age of each ram was determined and recorded

With the ram standing, both testes and epididimal anatomy were palpated and any abnormality in shape, size and evidence of injury or diseases were recorded. Scrotal circumference (SC) was measured at the time of doing physical examinations. It was measured by applying the measuring tape at the widest diameter of the testes.

Examination of the genital organs of the rams was applied while they were sitting up on their tail end except for the SC measurement, which was done at a standing position. The prepuce were examined for signs of injury, ulcers, mange and abnormal changes. Then the penis was extruded by grasping the sigmoid flexure firmly between finger and thumb and pushing upward toward the prepuce until the penis emeraged. It was examined for any inflammation, abscesses, adhesions, and abnormal deviation of the penis. The glans and the presence of the urethral process were observed. Animals that had severe health and physical problems were excluded.

Semen was collected using Bailey or Lane electroejaculators (Figure 3). The ram was put on his side and the penis extended and manually held with a piece of clean gauze during semen collection. The rectum was cleaned and lubricated then the electro ejaculator probe was inserted and 1-2 electrical stimulations were applied until semen was obtained. Color of the semen was recorded and the volume was obtained directly from the graduated collection tube. Semen motility and morphology were evaluated by using a light microscope following the Society for Theriogenology guidelines. Eosin nigrosin stain was used for morphology evaluation. Information was recorded and descriptive statistics obtained.

Figure 3: The two electroejaculators used in this study



Results:

Seventeen of the 82 Najdi rams (20.7 %) were affected severely by either health physical or genital tract abnormalities(table 4).

Table 4: Summary of health and physical severely affected Najdi rams.

SERIOUS PROBLEMS	Problem	Number of rams
Health and Physical	None	65
	Bad jaws or teeth	2
	Lameness	3
	Chronic mange	2
Genital	No urethral process	2
	Phimosis	1
	Swollen testes	3
	Firm testes	1
	Damaged penis (Balanoposthitis)	2
	Inguinal abscess	1
Total		82

The information in this table shows that approximately 58.8% (10/17) had problems associated with genital tract and 41.2% (7/17) with health and physical problems. In addition to these major problems, eleven rams had mange, eight rams had abscesses and one had diarrhea. . Fifteen rams had over grown toes and at the time of the examination, all the rams were not shorn.

The average age of all tested rams was 3.4 years and they had an average BCS of 3 and SC of 30.9 cm. Table 5. 6,7 and 8 show more details about age, BCS and SC measurements.

Table 5: the age, BCS and SC measurements

Parameters	Mean +/- SD	Range
Age (years)	3.4 +/- 1.58	1-6
BCS	3.1 +/- 0.43	2-4.5
SC (cm)	31.0 +/-1.95	26.5-35.5

BCS=Body condition score SC= Scrotal circumference

Table 6: category of Najdi rams ages

Age of the ram (years)	Number of rams	Percentage (%)
≤ 2	28	34.1
$> 2 - \leq 3$	13	15.9
$> 3 - \leq 4$	16	19.5
$> 4 - \leq 5$	14	17
$> 5 - \leq 6$	11	13.4
Total	82	

Table 7: Result of Body Condition scores (BCS) measurements

BCS	Number of Rams	Percentage (%)
≤ 2 – 2.5	22	26.8
> 2.5 - ≤ 3.5	56	68.3
> 3.5 - ≤ 4.5	4	4.9
Total	82	

Table 8: Result of scrotum Circumference (SC) measurements

SC (cm)	Number of rams	Percentage
≤ 27	2	2.6
>27 - ≤ 29	12	15.04
> 29 - ≤ 31	30	38.5
> 31 - ≤ 33	24	30.5
> 33 - ≤ 35	9	11.8
>35 - ≤ 37	1	1.3
Total	78*	

*SC of rams with injured testes were not included

All the rams were ejaculated after applying no more than two electrical stimulations.

Some ejaculated by using only one stimulation and semen was collected from only 70

rams. Rams that had serious genital problems and those with bad jaws or teeth were not collected.

Table 9: The number and percentage of rams with sperm abnormality

Abnormal cells (%)	Number of rams	Percentage (%)
≤ 30	50	67.4
<30 - ≤ 40	10	14.3
>40 - ≤ 50	9	12.9
> 50 - ≤ 60	1	1.4

Morphological analysis of the semen showed that separated sperm heads, bent tails and cytoplasm droplets were the major abnormalities observed in the semen. WBC's were observed in the semen from four rams, however, there were less than 5 WBC's per high power field (HPF). The serving capacity and libido were not measured in these rams.

Discussion:

Testing the potential fertility of rams is not a routine practice in Saudi Arabia. Most producers select their males based only on the appearance of the male without any consideration of the ram's potential fertility. Few breeders examine the reproductive organs and none evaluate semen . Neglecting male fertility tests is common in many countries. In the United States, recent surveys indicate that more than 40% of beef operations do not test the semen quality of bulls before use. In addition, the percentage of bulls subjected to semen and SC examination has decreased from 37.3 and 28.1 % in 1993 to only 31.1% and 17.7%, respectively in 1997 (4) .

At NADEC farm, the fertility of Najdi sheep is low, the average lambing rate is less than 50%. Selecting for potentially fertile rams could help increase the reproductive performance of this flock. This study showed that around 20% of the tested rams had serious health and/or reproductive problems and likely contribute to the low fertility of this herd.

Rams that had jaw and teeth problems could not feed as efficiently as normal rams and they exhibited low body condition scores. Their reproductive activity and semen quality would be decreased. Rams with chronic mange had low BCS and produced low semen volumes with very low sperm concentrations. These rams, if mixed with healthy ewes will , also be a source of spreading this disease.

Rams with abscesses and mange should not be used until they are treated. They should be separated from the healthy animals. Samples were obtained from the abscesses of four animals and diagnosed at the lab of the agricultural department. It was found that the etiologic agent of abscesses, caseous lymphadenitis, was *Corynebacteriaum pseudotuberculosis*. The incidence of abscesses in this farm in specific and in Najdi sheep in general is very high. Culling infected animals and vaccination may help decrease the infection rate.

Rams used were in various ages with 30% of the rams at least 5 years old. Using older rams is not recommended because they may have infections and be dominant, preventing younger rams from breeding. Old abnormal rams should be replaced by normal mature young males.

The measurement of BCS showed that 26.8% of the rams had a score of 2.5 or less. This is less than the recommended BCS that rams should have when used for

breeding (14). Males with condition scores of 2.5 or lower have little fat and may have some degree of muscle loss. This could affect their reproductive performance. Body condition indicates the amount of stored energy in these animals. As in cattle (29), low energy storage could decrease the release of GnRH form the hypothalamus which results in altered secretions of gonadotropin. This may affect the quality and quantity of semen produced by these animals. These rams must be supplemented with adequate balanced feed to improve their body condition. In this study, the size of the fat tail was used and it is helpful for scoring their body condition.

The percentage of rams with genital problems was 12% of the total ram population,but 58.8% of the number of rams that were suggested to be culled . The two rams who had no urethral process could have a reduction in their fertility. Phamosis is a defect which prevents the extension of the penis. Rams with this defect will not be able to breed any ewe. Rams that had swollen testes might be infected with *Brucella ovis* . No blood samples were taken from these animals to confirm this expectation.

The rams with firm testicles might have had pathological problems such as orchitis and thus will not be good candidates to be used for breeding. Rams that had a damaged penis will not be able to serve ewes. Hair rings that were found surrounding the penis of several rams might be the cause of this problem. Rams might get this hair while trying to breed unshorn ewes. The hair on the fat tail might have stuck to the penis and caused this inflammation.

Scrotal circumference (SC) is a heritable trait and it has a heritablity of 35% (30). Scrotal circumference measurement is highly correlated to testicular weight and is also directly correlated to the semen production and its quality. The average SC of this group

of Najdi rams was 30.98 cm. This is lower than the recommended measurement. It was suggested that the reason behind this low SC is due to the small size of this breed and because the measurements were taken at the beginning of the low breeding season. In this study, the SC measurements where not highly affected by the ram's ages (r=0.52) nor by the body condition (r =0.31). Selecting for large SC should be considered in this breed because of the advantages as has been described.

Semen evaluation showed that (7 rams)10% of the tested rams had less than or equal to 30% progressive motility. The reason of this low motility might be due to the elevated temperature of the environment, approximately 90F. In addition, the reduced body condition of these rams might add to this problem. By the time these rams were collected, they had not mixed with ewes for at least two weeks. They were collected only once and their first ejaculate showed this result of low motility. Bulgin (25) cited that the ejaculate order affected the percentage of dead and abnormal sperm and this ultimately affected motility. After a period of rest, first ejaculate can have less sperm motility than second and so on.

Semen quality not only has a relationship with fertility, but also with fecundity. For this reason, those 20 rams with greater than 30% abnormality should not be used until they are retested. Knowing the type of sperm abnormality will be useful in determining when the rams should be retested. High temperature, time of the year and the rest period could cause these abnormalities. Separated sperm heads, acrosomal damage, bent tails and cytoplasmic droplets were the common type of abnormalities seen in the ejaculate. Three of the rams with high abnormal sperm cells were six years old and this may contribute to the problem.

White blood cells (WBC) may be induced by illness and other bacterial infection, of the reproductive tract as well as by *Brucella ovis* infection. No blood or semen were tested for the presence of B. ovis.

The importance of testing rams prior to their use had been emphasized. The challenge is to convince producers in general and sheep owners specifically that BSE is an economic procedure and its costs will be more than covered by profits obtained by increased offspring numbers and by being able to use decreased male to female ratios.

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Chapter 2

Estrus synchronization and Artificial insemination

Introduction:

Successful sheep production requires efficient management techniques. The understanding of the reproductive patterns of sheep is very important for developing new methods which can increase sheep reproductive performance. Estrus synchronization and artificial insemination (AI) are two examples of useful reproductive techniques that can be used. By using these two methods, the effect of seasonality in sheep reproduction can be overcome. Artificial insemination can be done more efficiently only with synchronized ewes. It is very important to synchronize estrus in ewes that are cycling naturally or during the non-breeding season for optimum results with AI. When ewes are synchronized, AI can be done at a fixed time in relation to ovulation or by breeding them after estrus detection. Several methods of estrus synchronization and AI can be used and a variety of results have been obtained. Many factors affect the results of estrus synchronization and AI programs. Some of those are: time of the year, ewes age, health, body condition, breed, nursing , time from last lambing, AI technique, semen quantity and quality, inseminator expertise and semen handling.

Sheep in Saudi Arabia tend to have active reproductive periods which extend from September until March. Then, they start a period of low reproductive performance which starts from the end of April until the end of August. During this period, the day length is increasing and the ambient temperature is extremely hot. Therefore, the use of estrus synchronization and AI technologes may enhance the Najdi sheep performance

during this time of the year. In addition, by using AI, the problem of having fat-tails which affect the completion of natural breeding can be eliminated. A great deal of research has been devoted for AI in sheep in many countries around the world. In Saudi Arabia, AI in sheep has not been used. For this reason, one goal of this study is to evaluate the effect of using this technology on Saudian local fat-tail sheep during their low reproductive season.

Literature Review:

Seasonality:

At the out of season time, every aspect of life is affected not only the reproduction. In some region, changes are seen in the amount of rainfall, feed availability, humidity, photoperiod and the ambient temperature (1). The change of these factors can stop or reduce the reproductive activity of sheep and other seasonal animals. Most breeds of sheep have seasonal breeding patterns which are affected mainly by the length of daylight. In some regions and during the increase of day length, ewes show a restricted breeding season and rams don't. However, there are several variations in semen production and characteristics. In addition, the sexual behavior of the rams tend to be reduced during the non-breeding season (2). The most important factors that affect seasonality are photoperiod change, environment temperature and feed quantity and quality (1).

Photoperiod:

Light is transmitted by the eye to the pineal gland by way of different successive neural tracts within the brain (2). The pineal gland synthesizes and secretes melatonin hormone (5-methoxy-n-acetyltryptamine) in the blood plasma only at night. Melatonin regulates seasonal changes in various species such as sheep. It acts on specific receptors in the suprachia somatic nucleus (SCN) of the hypothalamus neurons which control lutenizing hormone (LH) secretion and ultimately reproductive activity (3). This fact was determined early in 1937 by Marshall, cited by McDonald (3). During the past 34 years it has been clarified that the role of the pineal gland is the regulation of photoperiod and its effect on reproduction in some mammals (4). Melatonin has an effect on the hypothalamic pulse generator and this elevates the synthesis and production of LH. Consequently, follicular growth is stimulated and the female ovulates (5). The first estrus cycle of the season may be shorter than normal and the first ovulation is not usually accompanied by estrus behavior. This is called "silent estrus" which occurs due to the lack of pre-ovulatory production of progesterone because there is no corpus luteum (CL). Progesterone is very important for full expression of normal estrus behavior (6). The administration of melatonin either by ingestion, injection or by constant release devices allow short days to be mimicked (7). The effect of melatonin in sheep is variable from breed to breed. Some breeds show longer breeding season such as Merino while, others have short seasons like Blackface and Southdown breeds. The length of breeding season of Merino is about 200 days versus only 120 days in Southdown. (8). In addition, it was found that female sheep are not only influenced by melatonin and photoperiod in postnatal life but also during prenatal life (7).

Environmental Temperature:

Seasonal variation of temperature plays a major role in the regulation of sexual function especially in tropical sheep (2). The temperature of the environment demonstrates marked effects on fertility, embryo survival and fetal development (9). Therefore, most of the northern hemisphere's breeds go to non-breeding season during summer months when the ambient temperature is high. The female sheep is affected by the increase of climatic temperature (2). It causes the ewe to be stressed and unable to maintain normal body temperature. Some factors can increase the effect of hot weather such as high humidity , activity during grazing, excessive body fat or having unshorn wool or hair. High temperatures was found to have an adverse influence on female fertility (9, 10, 11). In sheep, increase of the ambient temperature during days 10-15 of the estrus cycle decreases duration of receptivity or suppresses estrus behavior. In addition, it can increase cycle length and ultimately depress fertility (2). Most embryo wastage occurs when heat stress takes place between day one and 16 post breeding (2). Once the embryo has implanted, it will be less affected by thermal stress. However, the lamb weight at birth is negatively influenced by having high temperatures during the last half of pregnancy (2). The mechanism by which temperature influences on reproduction is not clear. However, Thatcher et al. suggested that under conditions of heat stress, blood is diverted to the skin and away from deep body tissues, such as the reproductive tract, in an attempt to maintain body temperature. This would alter the availability of hormones , nutrients and electrolytes to the uterus and oviduct and this could inhibit normal fertilization and early embryonic development (11)

High temperatures also affect male reproductive system. High temperature can lower semen quality and sperm motility (2). During prolonged periods of excessive hot weather, in summer, rams may temporarily become sterile. Abnormalities of the sperm that are associated with high ambient temperature appear generally in the sperm head such as: damaged acrosomes, pyriform heads, and separated heads. In addition, cytoplasmic droplets and bent tails can be observed in the ejaculate (2). Rams of some breeds are affected more by increase of ambient temperature than others. For example, Horned Dorset are more sensitive to elevated temperature than Merino rams (2). Many methods can be used to decrease the effects of high temperature on the animals. In Saudi Arabia, during hot dry summer months, the effect of shearing, shade and water sprays on the heat tolerance of Najdi sheep were studied (12). It was found that using these methods decreased the atmospheric temperature surrounding the animals. Utilizing these techniques will insure the animals' comfort which will be reflected on their production performance. It is very important to provide water , shade and improve the management during the hot months. In addition grazing early in the morning and late afternoon can prevent the animal from being exposed to the sunlight during the middle of the day.

Nutrients and Body Condition:

During the out of season period, in some places, the quality and quantity of feed is low. This is primarily due to the low level of rainfall and the increase of ambient temperature (1). Reproduction is an energy consuming process (1). Once the body demands of energy have been met, the excess calories can be used for other options such as supporting reproduction. This is true in females as well as in males. It has been thought for years that raising the amount of nutrition before and at the breeding season

can stimulate ovulation rate and this commonly was known as "flushing" (2). Underfeeding the female lamb during the first year of life can decrease the ovulation and twinning rates during her adult life (2). Moreover, low nutrients result in a cessation of estrus with suppression of ovulation in adult ewes. In Australia, a significant increase in ovulation rate (0.2-0.3 extra ova per ewe ovulating) occurred after six days of the start of lupin grain feeding to Merino ewes (2). In another study, ovulation rates were stimulated by increasing energy and protein in the diet (13). It was suggested that feeding ewes high protein diet caused an increase in liver weight and its portal blood supply. This might increase the breakdown of steroids in the liver particularly estradiol resulting in reducing the negative feedback of the estrogen on the hypothalamus and anterior pituitary. This will cause the release of gonadotrophins and finally an increase of ovulation rate (9). In addition, diet with low levels of nutrients can effect other aspects of ewes' reproductive performance such as: fertilization rate, embryonic mortality, mammary gland development, lactation and postpartum interval. Lambing rate and average lamb weight were improved when Najdi ewes were supplemented with concentrates (14). Rams are also affected by low levels of nutrition. Libido, testes weight, and the total number of sperm in the ejaculate can be adversely affected by low feed level (2).

Normal estrus cycle:

Estrus cycle is defined as the time between the periods of estrus. The ewes are considered as seasonally polyestrus breeders. During the breeding season, the ewe shows cycles of 16-17 days. Estrus duration ranges from 24-30 hours and ovulation occurs toward the end of estrus. The ewe shows only subtle signs of estrus such as : seeking the

male, standing to be mounted, vulvar edema and frequent urination at the presence of the male (15). The estrus cycle can be divided into two phases: follicular phase which includes two periods; pre-estrus and estrus, and luteal phase which includes metaestrus and diestrus. Luteal phase is longer, 14-15 days, than estrogenic phase which is only 2-3 days. Regression of the corpus luteum (CL) occurs on day 13 of the cycle induced by the hormone prostaglandin F2 alpha (PGf2alpha) which is secreted by the uterus (day zero is the estrus day). If the ewe becomes pregnant, the CL will remain until just prior to parturition. Females normally do not show estrus before puberty, during the non breeding season and post partum (2). Healthy well managed mature ewes should cycle regularly every 16-17 days during their breeding season.

Advantages of estrus synchronization

Estrus Synchronization has many potential advantages which include the following:

1. Gives the producer more than one lambing crop each year.
2. Shorter and earlier lambing season resulying in more uniform lamb crop and heavier weight at weaning.
3. Induce estrus at the time of anestrus which occurs during the non-breeding season.
4. Useful with AI because heat in sheep is difficult to detect so ewes can be inseminated at a fixed pre-determined time.
5. Economic use of labor.
6. Pregnancy examination can be carried out at an optimum time.

Keys to success in Estrus synchronization program:

A-Ewes must be:

1. Fertile
2. Disease free
3. Have good body condition
4. At least 30 days from last lambing.

B- Synchronized ewes are bred by fertile rams or artificially inseminated by an expert inseminator using good semen.

C-Select the right estrus synchronization technique.

D- Introduction of teaser rams at specific time on the synchronization can enhance the effect of synchrony.

E-Lamb removal enhances the response of synchrony.

Estrus Synchronization protocols:

A. Progesterones:

Progesterone is a steroid hormone that is mainly produced by the luteal cells of the CL. Progesterone is transported in the blood by a binding globulin. Progesterone secretion is primarily initiated by LH. This hormone prevents the ewe from showing estrus and helps in preparing the reproductive tract for receiving the fertilized egg and later for the maintenance of pregnancy. It is possible to mimic a CL by using natural or synthetic progesterones. Progesterone products (progestogens) are usually given for a period of 12-16 days, either as subcutaneous implants, vaginal devices, or orally with the

feed. When progesterone product is withdrawn from the female, there will be an immediate fall in its level in the blood circulation, closely mimicking what happens naturally when a CL disappears. When the concentration of progesterone decreases in the circulation, this will allow an increase in hormones such as gonadotrophin releasing hormone (GnRH), follicle stimulation hormone (FSH), and LH. The increase of these hormones' levels causes follicular development and ovulation. Using progestogens alone will not be sufficient for estrus introduction especially in the non-breeding season. Therefore, gonadotrophins must be given at the time of progesterone removal. Many progestogens can be used for estrus synchronization in sheep. Following are some of these methods:

1- **Norgestomet Implant (Synchro-Mate-B):**

Synchro-Mate-B is an ear implant containing synthetic progesterone, norgestomet. These implants are used for heifer synchronization but work well for synchronizing ewes. A single synchro-Mate-B implant contains 6 mg norgestomet. In cattle, these implants are used in combination with an intramuscular injection of 5 mg estradiol valerate and 3 mg norgestomet. This injection is given at the time of implantation to take account of cattle that are either in the early or late stages of their estrus cycle. In sheep, the implants work well without need for this injection because they are more sensitive to estrogen (24). Only one half implant (3mg) is used per ewe. The implants are inserted SQ in the ear with a specific gun and left in place for 10-14 days. The implant should be inserted deeply subcutaneously in the back of the ear to avoid their loss. The advantages of using norgestomet implants include: 1) The loss rate is very low; 2) No vaginal discharges occur as are seen when vaginal sponges are used,

3) No vaginal trauma or discomfort occurs as with vaginal methods and 4) Good results are achieved. On the other hand, there are some disadvantages such as: 1) These implants are expensive when compared with other methods and 2) require a simple surgery for removal.

There are many published studies where norgestomet implants were used. Ainsworth (18) cited that norgestomet implants were used early by Martin et al., Goboland and Spitzer in 1976, 1979, and 1980 respectively . In these studies, norgestomet implants gave encouraging results for controlling estrus and ovulation in sheep. Ainsworth et al. obtained 96% synchrony when they implanted crossbred ewes with 3 mg norgestomet implants which were left in for 12 days and 500 international units (i.u.) pregnant mare serum gonadotrophin (PMSG) was injected intramuscularly (i.m.) at the time of implant removal (18). Martinez studied the effect of giving injections of norgestomet at the time of norgestomet implantation (19). In his study, twenty eight cycling Pelibuey ewes were used. The ewes were either synchronized by only norgestomet implant (group 1) or with a combination with two injections of norgestomet, 1.5 mg, and estradiol 2.5 mg at the time of implant insertion (group 2). The ear implants were left for 11 days in both groups. One hundred percent of ewes in these two groups showed estrus 60 hours post removal. The majority of ewes, 80%, in group one exhibited estrus behavior within 36 hours of the implant removal versus only 30% in the second group. The conception rates after synchronization were 70% and 80% in group 1 and 2, respectively. No significant difference between group 1 and 2 in regard to the litter size 1.29 versus 1.25, respectively, was observed.

The effect of PMSG injection at the time of implant removal was tested in 60 Dorset and Rambouillet x Dorset ewes (20). Half of the ewes were treated only with norgestomet implants for 10 days and the other 30 ewes were treated as the first group plus injections of 500 i.u. PMSG at the time of implants removal. The percentage of ewes exhibiting estrus were higher in the second group 70% in comparison with the first group, 65%.

Induction of estrus at the non-breeding season was examined by several researchers. Yelich et al. used 141 ewes that were not pregnant at the end of their normal mating season (21). The ewes were divided into two groups: the first group (95 ewes) were implanted with norgestomet implants for 7 days and injected with 400 i.u. PMSG plus 200 i.u.. hCG at the time of norgestomet withdrawal. The second group (46 ewes) served as untreated controls. Pregnancy rates were 44.3% and 6.6% respectively. In another study (22), ewes were implanted with 2 mg norgestomet for 14 days and given 500 i.u. PMSG at the time of implant removal. Ninety six percent of the ewes exhibited estrus. The pregnancy rate and the number of lambs born per ewe lambing were 60% and 1.7, respectively. Lopez et al. used norgestomet implants to induce estrus in 90 cross bred March ewes which were synchronized either during their breeding season or at the non-breeding season (23). Ewes were implanted with 1.5 mg norgestomet for 12 days and injected with 500 i.u. PMSG at the time of implant removal. The pregnancy rate was 38.3 % for the ewes treated in their breeding season versus 36.6 % in ewes treated at the time of non-breeding season.

The time of ovulation after norgestomet implants was evaluated by Gardwell et al. (20). Ewes were treated with norgestomet alone or in combination with PSMG injection.

Ovaries were monitored by using rectal ultrasonography every 6 hours to determine the interval from implant removal to ovulation. The results showed that ewes ovulated an average of 79.8 and 46.0 hours after implants removal in the first and second groups respectively.

Table 1 summarized the results of other studies where norgestomet ear implants were used.

Table 1: (this table is taken from Wildeus, 1993, (24)

Dose (mg)	Duration (days)	Associated treatment	N	% estrus	Mating system	% fertility
2	14	PMSG 500 i.u. At implant removal	128	93	Natural	61
1 1.8 2	12	PMSG 500 i.u. at implant removal	107 107 107	90 93 97	Natural	65 84 79
3	14	FSH-P 6mg,8 injections, twice daily,0 hours of implant removal	25	48	Natural	40
6	9	PMSG 320 i.u. at impl. removal.	131	90	Natural	55

2) **Melengestrol Acetate (MGA):**

Melengestrol acetate (MGA) is an orally administered synthetic progestogen that was developed for cattle and can be used for synchronizing ewes during and out of season (24). It is the most widely used type of progesterone that has been used for synchronizing heifers. It has some good features that make it commonly used. Some of

these advantages are: 1) it is not expensive, 2) it gives acceptable results, 3) It induces estrus and allows the timing of insemination and 4) it is easy to use. However, it has some disadvantages that include: 1) in cattle, because of the long administration period, it can increase the number of atretic follicles, 2) The amount of the hormone that is consumed by an individual animal can not be practically controlled and 3) it may affect the transportation of sperm in the ewe reproductive tract.

The first time MGA was used was in the mid 1960s where it was administered orally and it had growth promoting action and at the same time the capability of inhibiting estrus in cattle (16). It can be mixed with feed rations and fed twice a day. Wildeus (24) cited that Keisler and coworkers developed dosage and treatment schedules for the use of MGA in sheep in conjunction with both estradiol, mainly zeranol, and gonadotrophins , usually PG 600 (400 i.u. PMSG + 200 i.u. hCG). In addition, Wildeus mentioned that the results of using MGA were not consistent and higher doses appeared to depress fertility and estrus induction.

The use of MGA to synchronize estrus in ewes was evaluated by many researchers. Goel et al. , in 1989 , treated a group of Mozaffarangari ewes with a low amount of MGA, 0.15 mg per day for 15 days (26). Approximately 84% of the ewes exhibited estrus behavior after MGA removal and 81.9% of them became pregnant. In another more recent study, Quispe and coworkers, examined the use of MGA for synchronizing cycling ewes (27). Ewes were fed 0.22 mg MGA daily for 14 days. They found that 79% of treated ewes showed estrus versus only 37.5% of the untreated animals.

Several trials have been conducted using MGA during the non-breeding season. Umberger and coworkers did several experiments using different amounts of MGA. In the first study , 35 ewes were given 0.125 mg MGA twice a day in the diet for 10 days and 31 untreated control ewes (28). Lambing percentage was 68.6 % in group treated ewes versus 58.1 in the control group. In addition, 95.8% of the treated ewes lambed in the first 30 days of the lambing season compaerd to 61.1 % in the control group. In another study, Umberger and coworkers did an experiment (29) where 140 anovulatory Dorset, Suffolk and Hampshire ewes were randomly assigned within group breeds into four different treatments: 1) the untreated control group, 2) MGA only , 3) MGA and PG600, 4) Synchro-Mate-B and PG600. The Synchro-Mate-B implants, (3 mg) were left for 10 days. One half of the ewes in each group were placed with fertile rams equipped with marking harnesses at the end of their hormonal treatments. The results indicated that ram exposure or treatment with gonadotrophin were very important for getting a high percentage of estrus induction in the non-breeding season. In another study, Safranski et al. fed ewes with 0.125 mg MGA twice a day for 9 days in one group and used the same treatment plus an injection of 5 ml PG-600 in the other group (30). The PG-600 group was injected at the last day of feeding MGA. They concluded that all these treatments increased the number of ewes exhibiting estrus. However, in this study, PG-600 did not increase ewe's productivity over that achieved using MGA alone.

3) **Progestogen pessary or sponge:**

These devices are intra-vaginal sponges that are impregnated with synthetic progestogen. Up until 1964, progesterone was administered oraly by either daily injections or by feeding (31). In 1965, these intravaginal devices were developed in

Australia by Robinson and associates (32). There are two different types of sponges. Maxwel et al. , explained in detail the difference between these two types, the way they work and how they can be used (33). The two kinds of sponges are : 1) Chronogest which contains 30, 40 or 45 mg Fluorogestone acetate (FGA) and 2) Repromap which contains medroxy progesterone acetate (MAP). There is no difference between these two types of sponges when natural breeding or high dosage of semen are used. However, when ewes inseminated with low numbers of sperm cells, the fertility was lower when MAP (60 mg) was used than when FGA (30 mg) was used (34). The cause of this reduction in fertility might be due to the higher amount of progestogen in MAP which could affect the transportation of sperm in the female reproductive tract. The sponges are inserted into the vagina with the aid of a specific applicator (33). When the sponges are inserted, progestogen will be slowly released and absorbed through the wall of the vagina and into the blood stream. Sponges are usually left in the ewe for approximately the same time as the CL is normally present on the ovary, normally 12-14 days. Pregnant mare serum gonadotrophin (PMSG) injection is needed when it is desired to synchronize ewes during the non-breeding season.

The use of the pessaries gives good results and allows timed inseminations (50-60 hours after their removal). However, common complications (17) such as:

1)- Treated ewes can develop a severe vaginitis when sponges and equipment are improperly handled or lack lubrication.

2)- The fall-out rate is between 2-25%.

3)- Insertion of sponges may result in mucosal injury and adhesion formation especially in maiden ewes.

4)- Using sponges may cause abnormal vaginal discharge which sometimes has very bad odor.

Sponges have been used in sheep for more than 30 years (34). In 1976, Le Roux treated 45 Karkal ewes with 40 mg MAP sponges which were inserted for 15 days. Ewes received 400 i.u. PMSG on day 13 (35). The ewes were bred naturally or artificially. The conception rate was 68% after natural insemination and 57% following AI. Dyrmundsson, 1977, treated 738 Iceland ewes with 60mg MAP during the normal breeding season, late November and early December (36). The sponges were allowed to remain for 14 days. The ewes were injected with 500 i.u. PMSG at sponge removal and at this time were either placed with fertile rams or artificially inseminated 48 hours post t sponge withdrawal. Pregnancy rate was 90% in naturally bred ewes versus 65% in the AI group.

Robinson and Coworkers studied the effect of injecting different level of PMSG on the fertility of ewes synchronized by FGA(37). The FGA sponges, 30mg, were inserted intravaginally in four groups of ewes and left for 12 days. At the time of pessarie removal, ewes were injected with 250, 500,700 and 1000 i.u. PMSG in group 1,2,3,and 4, respectively. The results showed high percentages of the ewes in all the four groups exhibited estrus behavior 89.3, 96.4,100, and 77.6%, respectively. However, the fertility rate was low 22.6, 25, 36.9,and 35.7 in groups 1,2,3,and 4 respectively.

4)- Controlled Internal Drug Release Devices (CIDRs)

Controlled internal drug release devices (CIDRs) are solid devices which impregnated with natural progesterone. They are an intravaginal pessary that were developed in early 1980's in New Zealand by CHH Plastic Molding Company in

conjunction with the Ministry of Agriculture and Fisheries (38). These devices have a specific applicator where they can be loaded and inserted into the vagina. The CIDR's are good devices that can be used for estrus synchronization in sheep. There will be no fluid discharges from the vagina at their withdrawal. The interval from their removal to ovulation is about 8 hours earlier than when sponges are used (39,40). In addition, they can be reused and their insertion and removal are very easy (7). However, in some studies, a high percentage of losses was were reported (41).

The use of CIDR's has been evaluated in many countries. In New Zealand, Lowe et al. , used CIDR in combination with PMSG to synchronize Romney ewes. The pregnancy rate of the treated ewes was 71% (42). In Canada, it was found that the insertion of a CIDR for 14 days in ewes was as effective for estrus synchronization as insertion of FGA (43). A high percentage of ewes in each group (CIDR, 92%, and in FGA, 91%) were marked by the ram within 72 hours after withdrawal of treatment. A recent study, 1999, in the tropics, has shown that the use of CIDRs has given 100% estrus synchrony after 36 hours of their removal (44). In this study, teaser rams were introduced to make the ewe show estrus earlier and closer to each other. In this study, a reasonable conception rate, 52.9%, was achieved when ewes were inseminated artificially by trans-cervical technique.

The use of CIDR in comparison to other intravaginal devices were examined in many studies. Crosby et al., 1988, evaluated the use of four intra-vaginal devices combined with varying levels of PMSG in anestrus and cyclic ewes (45). They used FGA, MAP, CIDR, and sponges contains 500mg progesterone. They conclude that ewes that were synchronized with CIDR had higher lambing rate (80%) during the

anestrus season. In the cycling ewes, 88% of the ewes in the CIDR group exhibited estrus, but had the lowest conception rate (56%) amongst the treatments. In another study, Rhodes et al., compared the use of CIDR that containing 366 mg progesterone with MAP containing 60mg progestogen during the breeding season (46). No PMSG was used in this study. They found that these two devices gave reasonable results of synchrony. In addition, ewes receiving CIDR succeded in showing estrus earlier and with closer synchrony than those received MAP.

B-Prostaglandins:

Prostaglandin PGF2 alpha (PGF2a) is one of the arachidonic acid products. PGF2a and its analogues have been shown to be effective luteolytic agents in sheep and other species. The mechanism of PGF2a action is to induce regression of the corpus luteum (CL). in cycling ewes. PGF2a is secreted from the uterus on day 13 or 14 and cause the end of an estrus cycle and the beginning of a new cycle. Under normal , seasonal season, conditions, approximately 6% of the ewes will be in heat on any given day Therefore, when a single PGF2a injection is given, around 70% of the ewes will exhibit synchronized estrus 30-48 hours from the treatment. The thirty percent of ewes that don't show estrus are between day one and five of the cycle and they either don't have CL or are developing a new CL which is not affected by the PGF2a . When two injections of PGF2a are given 9-11 days a part, all the treated ewes will show estrus after the second injection (24).

Natural and synthetic prostaglandins are available and can be used for estrus synchronization during the breeding season. The common natural form of PGF2a is (Lutalyse®) and synthetic prostaglandins include; cloprostenol (Estrumate®) and

prosolvin (Intervet). All of these prostaglandins are available in injectable form. The synthetic PGF2a is more potent than natural form.

The advantages of using prostaglandin products include the following: they give good results and allow fixed time insemination. However, they are expensive and their effect depend on the presence of an active corpus luteum. Therefore, they cannot be used for induction of estrus, during the non-breeding season.

Many trials have been done using PGF2a or its products. Fuki and Robert (1977) conducted an experiment to investigate the fertility rate in ewes that were synchronized by either one injection or two injections with an interval of 12 days. They found that there was no significant difference in results of estrus synchronization following one injection, (74%) or when two injections, 72%, were used. They also found that the fertility rate was the same in these two treatments (47). In another study, Fuki, found that using PGF2a gave good results and there was no influence on the transportation of the sperm at the female reproductive tract after the use of PGF2a as had been described before (48). A study by Hoope and Slyter (1985) also showed no difference between one and two injections (49).

The period between two PGF2a injections affects synchronization and the lambing rate when the period between two injections is 5 days. On the other hand, it is not economical to extend the period for two injections. Fairrue et al. , in 1978, treated Merino ewes with two injections of cloprostenol (50). The length between treatments was varied, 12,13,14,and 15 days with resulting lambing rates 14,41,55, and 55%, respectively.

The difference between using synthetic versus natural PGF2a for synchronizing estrus cycle was evaluated by Acritopoulou et al., 1982, (51). They gave two injections of synthetic or natural PGF2a to Karagoumko and Serrai ewes. They concluded that synthetic PGF2a gave less lambing rate, 32.5% than natural PGF2a, 60%. However, the number of lambs born per ewe was higher when synthetic, 1.53, than when natural, 1.33, were used.

Mutiga and Mukasa, in 1992, compared the effect of using two methods of estrus synchronization (52). They used 72 mature cycle Menz ewes. They gave either 2 injections of PGF2a given 12 days a part or vaginal progestogen sponges for 12 days. With each treatment, they gave different levels of PMSG, 0, 200, or 300 i.u. The results showed that ewes treated with PGF2a exhibited estrus earlier than those treated with progestogen sponges. They also found that the increase of PMSG dosage was associated with an increase in twinning rate.

C- Melatonin:

Melatonin is a hormone that is synthesized and secreted by the pineal gland in response to darkness. The increase of melatonin , when the day length is decreasing, enhances the reproductive activity in seasonal sheep. Synthetic melatonin is a safe and readily available product. This hormone can be administered to ewes by either an orally active compound, injection, subcutaneous implants or intravaginal devices. Melatonin treated has no effect on ewes already cycling. It does not synchronize estrus in ewes, but if used with other treatment such as progesterone, synchronization can be obtained.

The induction of estrus by melatonin was tested by many workers. Forcada, Zarazaga, and Albeca, in 1995, found that implanting ewes with 18mg of melatonin

32days after lambing and at the beginning of the anestrus season, decreased the interval from weaning to first estrus by approximately 36days for treated ewes compared to untreated ewes (53). Stellflug et al., studied the effect of exogenous melatonin on reproductive performance of 737 Polypay and Crossbred-ewes. The ewes either received 2 or 10 mg in the feed or were implanted with melatonin for 40 days. They concluded that feeding or implanting melatonin for this period enhanced reproductive performance of mature ewes and overcame the effect of seasonality (54). Slyter and Weiskircher, divided 83 ewes to take one of four treatments: 1)- Natural day light (control), 2)- First treatment; natural day light plus melatonin (Regulin) implant, 3)- Second treatment: natural day light plus 3.5mg melatonin/day in their feed, and 4)- third treatment: 8 hours of light and 16 hours of darkness per 24h period (55). The treatments extended for 30 days, then, fertile rams were introduced. The lambing percentages were 74.6, 92.5, 88.1, and 95% in group 1,2,3, and 4 respectively. This treatment also resulted in an earlier lambing date. In another study, it was concluded that feeding Suffolk ewes daily melatonin, for 40-90days from early or mid anestrus season, increased the incidence of estrus when joined with fertile rams to 100% versus untreated ewes, 32% (56). Melatonin treatment also has been used to enhance the reproductive performance of rams (57)and for obtaining early puberty of ewe lambs (58).

D- <u>Natural Synchronization (Ram effect):</u>

Natural synchronization depends on ewes isolated from sight, sound, and smell of rams for at least 30 days. When the anestrus ewe is re-introduced to a male after the isolation period, her hypophyseal pituitary axis is stimulated. Then, the ewes gonadotrophin FSH and LH are increased as result of male presence. Ewes normally

will ovulate two to three days after male introduction and this first ovulation is not associated with estrus behavior (59). The ram effect is not effective when ewes are cycling or in a deep anestrus. The ram effect will be more increased if a highly sexual ram is used. Fitzgerald and Perkins discussed the importance of choosing rams that are very sexually active to improve the fertility of the flock (60).

The ram effect will be most effective if the ram itself is introduced to the ewes after a period of isolation. If only one of the ram's three factors, sight, smell, and sound are used, the production of ovulation can be low (61). The effect of ram introduction associated with progesterone were evaluates by many researchers. Fuki and coworkers, in1986, studied the effect of progesterone treatment associated with ram effect on the estrus induction and lambing rate in seasonally anestrous ewes. Ewes that were introduced to rams showed estrus earlier than those which were not introduced and the lambing rate was higher for introduced ewes (62).

The body condition of ewes at the time of ram introduction can increase response of ram effect. The influence of ewes body condition on ovulation rate when rams are introduced was evaluated by Lassored and Khaldi. A hundred percent of ewes with good body condition ovulated within 4 days of the introduction versus 93.3 of ewes with poor condition (63). In addition to the ram presence, the sexual activity, libido, may be very important in initiating ovarian activity (64). The ratio of rams to ewes was also found to be very important factor for optimum response of the ram effect. When Dorset rams were used at ratios of 0,3,6,and 9 per 100 ewes, this resulted in 0,72,81,and 86% respectively of ewes ovulating at the silent estrus (65). Finally, the introduction of rams

to young ewe lambs could advance their puberty onset and affect their ovulation rate (66).

Artificial Insemination :

Introduction:

The main objective in animal breeding is to improve their production by selection of individuals with highest breeding values as parents of the next generation. The use of artificial insemination (AI) can enhance the male side of selection especially if the best males are selected very carefully. The use of AI also insures the wide use of the superior rams. For this advantage and others, the Russian sheep industry early utilized this technique to improve the production of their sheep. The yearly number of ewes that were artificially inseminated reached 42-44 million (67).

The earliest reports on AI reach back into the middle ages. In the fourteenth century, an Arab inseminated his mare by means of pledget of cotton from vagina of a mare recently bred to a famous stallion and he inserted this cotton into the vagina of his own mare which became pregnant (68). The spermatozoa were first observed by Leeuwenhoek and his student Johan Hamm in 1677, with the aid of a magnifying lens. The first scientific research in AI of animals was performed in 1780 by an Italian physiologist, L. Spalanzani. He found that the fertilization power of semen was in the sperm which are swimming in the spermatic fluids (67). He had obtained success with several amphibious animals, then he decided to experiment AI in domestic animals. He started with a female dog which was inseminated with fresh semen and gave birth to three puppies. Spalanzani also, in 1803, contributed to the knowledge of the effect of cooling on the sperm cells (69). He concluded that when stallion semen was frozen in

snow or winter cold, the sperms stopped their movement and they re-established their motion when exposed to warmth. Rossi, in 1782, did some experiments which confirmed what Spalanzani had discovered. In 1884, around 19 female of dogs were inseminated artificially and 15 of them produced Young. (68). At that time some AI in horse was reported. Ivanoff was the first one to use the words, artificial insemination, in 1899-1930. He also, reported successful results of AI in cattle, horses, and ewes in 1907. In the early 1900s, Russian and Japanese researchers were active in doing work with AI. After the First World War, the AI research and application increased greatly in Russa (68). The number of animals inseminated was more than 16 million at that time. AI was first used in horse breeding in Europe in 1890 by Repigret, a French veterinarian. In the United States, the first administration of AI was in cattle at the Agricultural Experiment Station in Minnesota. The discovery of the practical method of semen freezing was obtained by Polge, Smith, and Parkes in England in 1949 (67). This discovery allowed semen to be preserved for long time and be used throughout the year.

Artificial insemination has not been used very widely in the sheep industry, but there has been a growing interest in recent years (67). The poor fertility which has been obtained especially when frozen semen is used may be associated with this low use of AI in this industry. In addition, the anatomical structure of the ewes' reproductive tract makes non-surgical deposition of the semen into the uterus very difficult. In recent years, improvement of freezing ram semen and the use of trans- cervical AI technique can encourage many breeders to use this technology.

Advantages of AI:

Many advantages can be obtained if AI is used. Some of these advantages include the following :

1)- In normal flock breeding, a ram can serve in average 50 ewes in the season. However, thousands of ewes can be bred from the same superior ram if AI is used.

2)- When using AI, there will be no copulation, therefore venereal diseases cannot be transmitted among the flock.

3)- Easy transport and introduction of genetic material without isolation and quarantine.

4)- AI permits reproduction when males are unavailable for natural mating due to many reasons such as death, aging, injury, or during the non-breeding season when the libido and the semen quantity and quality are low.

5)- If semen is preserved by freezing, semen of superior rams can be used long after they become very old or even after their death. Moreover, frozen semen can be shipped and used in other countries.

6)- When estrus synchronization programs are employed, extra rams are needed to breed the ewes which will come to estrus in short period. AI can reduce this requirement.

7)- Poor semen production is only detected if BSE is used or when it is too late. Therefore, using AI insures using adequate numbers of high quality sperm.

8)- More efficient crossing between different breeds. Ewes and rams from some breeds may not accept mating with other breeds due to differences in size. Using AI reduces the incidence of injury if females from small breeds are bred by very large rams from different breeds.

9)- Overcomes the low conception rate which occurs in fat-tailed sheep when natural mating can be impaired by the large fat-tail.

10)- Predetermining the sex of the offspring by using the technology of separating sperms carrying Y from X chromosomes.

11)- By using AI, the sheep owner can keep better breeding and performance records.

Artificial Insemination techniques :

Several methods have been used for inseminating ewes: vaginal, cervical, laparoscopic, and transcervical (table 3) (70). Here the focus will be on the two most common AI methods which are recently used for inseminating ewes.

Table 3: Methods of AI in sheep (Mylne, 1997)

Type of semen	Dose of p sperm	Location of AI	Expected lambing %
FRESH	400×10^6	Vaginal	20 – 60
	200×10^6	Cervical	40 – 80
	20×10^6	Laparoscopic	70 – 100
	100×10^6	Transcervical	40 – 100
FROZEN _ THAWED	400×10^6	Vaginal	5 – 20
	200×10^6	Cervical	-
		Synchronized estrus	25 – 40
		Natural estrus	30 – 60
	20×10^6	Laparoscopic	40 – 80
	100×10^6	Transvaginal	30 - 70

1)- Laparoscopic Intrauterine technique :

This technique insures the deposition of semen directly into the uterus. It became practical in the early 1980s and has been used for several years in Australia and New Zealand (71). This technique was developed in Australia in the early 1980s. Laparoscopic AI was reported from New Zealand in 1984 by Tervit et al. A low amount of semen was used and a 83% lambing rate was obtained (72). Many factors affect the success of this AI technique. Timing of AI is one of the very important factors that can affect the result. The time of insemination depends on whether the semen is fresh or frozen. In one study, fertilization rates with fresh semen increased from 71% to 97% as the time of intrauterine insemination increased from 24 to 48 hours after the removal of progesterone treatment. However, with frozen semen, a high fertilization rate was obtain when the AI was done, 48 to 55 hours from progesterone sponges removal (73). In another study, when frozen semen was used, no significant effect of timing was found if ewes were inseminated 48,60, or 72 hours post removal of the sponges. However, in this study, the type of vaginal sponges affected pregnancy rate. Animals synchronized with FGA had higher pregnancy rate than those synchronized with MAP (74). The methods of storing semen can also affect final fertilization rates as reported by Hunton et al. (75),and Maxwell (76). Laparoscopic AI technique has been explained in many articles (70, 71, 77).

The laparoscopic technique gives good results if fresh or frozen semen are used and it offers an economical use of semen. On the other side, the cost of this procedure is high and is ideally done at a specific locations where the laparoscopic and support

equipment are located. In addition, this method requires a minor surgery to make two small incisions in the abdomen which may concern animal rights group.

Transcervical AI (TC AI):

Transcervical AI was developed in Canada in 1988-1990, as an alternative of laparoscopic AI.. The researcher team at Guelph University, in 1988, designed the transcervical technique which is a humane, non surgical and very effective means of depositing semen directly into the uterus. The same team, later, developed a system of freezing ram semen. The TCAI method mainly consist of three parts: a simple system for holding the ewe to be bred easily, a speculum with grasping forceps to stabilize the reproductive tract and an instrument for handling semen and inseminations (70). A few years later, workers at Colorado State University modified the insemination gun to allow the inseminator to penetrate the cervix; then thaw and load the semen into the inseminating gun (78). This would reduce the interval between thawing and using the semen.

The TCAI is less expensive, more humane, less technically complex and can be conducted in the field without need for an equipped room. However, the major factor that limits use of this technique is rate of cervical penetration. Windsor, 1995, mentioned few factors which might affect penetration rate (79). He found that penetration rate would increase when ewes had either recently lambed or were in their natural breeding season and came to heat naturally. There are some other factors which have been shown to affect success of passing the cervix and ultimately the lambing rate. Some of these

factors include: period since last lambing and maybe the breed of the ewe. The steps of TCAI were explained by Mylne et al. (70), and they include the following:

1)- The ewe first restrained in dorsal position by using the commodore foot trimming cradle.

2)- Insert the plunger inside the speculum and lubricate the tip of plunger and gently insert them into the vagina with a slight rotating movement. After the insertion, the plunger will be taken out.

3)- Insert the light source into speculum and try to locate the cervical opening and if there is an excessive mucus, it should be removed.

4)- When the cervical opening is observed, a forceps is used to grasp it which is a very important step that can affect penetration ease.

5)- Find the entrance to the cervical canal with the tip of the insemination gun and gently guide it through the cervical rings until you feel that the tip is in the lumen of the uterus. This is the most critical step which requires experience and patience.

6)- Load the semen to the gun and expel it very slowly.

Trials using Laparoscopy and Transcervical AI:

Davis et al. In 1983 evaluated the laparoscopic technique. They inseminated synchronized ewes with different doses of spermatozoa ; 12.5, 25, 50, or $100X10^6$. Ewes were inseminated 50-56 hours after the removal of progestoge vaginal sponges. The pregnancy rates of inseminated ewes, that showed estrus were 75,77,77,and 76, respectively (80). Taljaard et al., studied the effect of laparoscopic insemination on the estrus cycle of 31 Dorper ewes. Their conclusion was that this technique did not affect

the length of the estrus cycle (81). A non-surgical intrauterine insemination was used before the Guelph technique was reported. This early study of using non-surgical intrauterine insemination was done in 1977 by Fukui and Roberts (82). They conducted an experiment to examine the repeatability of non-surgical penetration into the uterus through the cervical canal. The aim of this study was to find a way for non-surgical intrauterine insemination. They obtained 76.6% success of cervical penetration. One of the earliest evaluations of the Guelph transcervical AI was done by Halbert et al., in 1990 (83). They compared the pregnancy and lambing rates with ewes inseminated transcervically or laparoscopiclly. The pregnancy rate was 55% and 1.2 +/- 0. 2 lambs born per ewe inseminated when TCAI used. On the other hand, ewes inseminated by laparoscopic technique had higher lambing rate, 65%, and more lambs born per ewe inseminated, 1.5 +/- 0.2. The Guelph TCAI technique gave best results when it was used during the breeding season and performed on ewes with recent lambing. In addition, inseminator experience was essential for higher cervical penetration which led to a successful insemination (84). Sayre and Lewis examined the effect of exogenous oxytocin treatment on cervical dilation and sperm movement into the oviduct in ewe (85). They concluded that oxytocin treatment induced cervical dilation and this could facilitate TCAI in sheep. This study also showed no effect on sperm movement throughout the uterus and into both oviducts when oxytocin was used. Insemination of ewes after estrus detection or by time fixed insemination were evaluated by Husein et al. . They used TCAI to inseminate 25 ewes that were synchronized with sponges or controlled internal drug release device-type G (CIDR-G) (86). The ewes were inseminated 8-28 hours following onset of estrus with approximately 245 X 10^6 motile post thawed spermatozoa.

The conception rate was 47% based upon progesterone profiles and ultrasonography. In another study, a fixed time insemination with post thawed frozen semen was used (87). The ewe weres synchronized with CIDR for 14 days during their breeding season. Then, the ewes were inseminated 50-56 hours after the CIDR removal. Ewes were inseminated either by laparoscopic or TCAI techniques. The pregnancy rate was higher with laparoscopic insemination 63% than with TCAI 40% (87).

ESTRUS SYNCHRONIZATION AND AI OF NAJDI SHEEP:

Materials and Methods :

Experiment Design :

A clinical trail was conducted at the end of spring, 1999, when Najdi sheep show low reproductive activity. All Najdi rams used were previously given a breeding soundness examination including a detailed semen evaluation. Three hundred Najdi ewes were allocated equally to three groups:

Group 1(control ewes):

Ewes in this group were not given any hormonal treatment and were placed with 8 fertile rams for 17 days.

Group 2: (Treatment 1)

Ewes in this group were synchronized with CIDR-G which contains 330mg natural progesterone. The CIDR's were inserted and removed after 12 days and at the time of removal, an injection of 600 i.u. PMSG was given to each ewe, and 10 fertile rams were placed with them for 7 days.

Group 3: (Treatment 2)

Ewes in this group were treated similar to group 2 in regards to hormonal treatments. However, at the time of CIDR removal, 5 teasers were introduced and ewes were inseminated trans-cervically 48-54 hours of the removal. Ewes were inseminated with $100X10^6$ extended fresh Najdi semen.

Operational Hypothesis :

The Null Hypothesis : The percentage of pregnancy in group 1, 2, and 3 is the same.

The Alternative Hypothesis: At least one of these three groups is different in pregnancy percentage.

Animals:

Najdi ewes that were used in this study were examined at the beginning of this study. The identification ear numbers, ages, BCS and any sign of health problems were recorded. Ewes with missing ear tags received new ear tags. The ewes ages were determined by checking their teeth. Ewes were given a subjective body condition score (BCS) from 1 to 5 where very thin and very fat animals were given score of 1 and 5, respectively. Only ewes that had BCS of 2.5-4 were included in this study. Ewes with health problems were excluded. All of the ewes had lambed recently and they were from 45-60 days of their lambing. At NADEC, the weaning takes place approximately 45 days from lambing. Therefore, by the time these ewes were used, the ewes had been separated from their lambs from 1-15 days.

Ewes selection:

Three hundred Najdi ewes were selected for this study. All the ewes were run into a long chute and were marked with a different color on the head. Red, blue, and

green head marks were given to the first, second, and third animal in the line , respectively, and the same marking system applied to the rest of the ewes. Group 1,2,3 included animals that was marked with red, blue, and green head marks, respectively. The ear tag number of each animal was also recorded.

Estrus synchronization :

Ewes in group 1 were not synchronized. Ewes in group 2 and 3 were synchronized by inserting CIDR-G devices which were lifted for 12 days. At the time of CIDR's withdrawal , each ewe was injected with 3ml pregnecol containing 600 i.u. PMSG.

Pregnecol (Figure 4):

Pregnecol (Figure 4) is an intramusculor injectable brand of PMSG that is manufactured in Australia by Horizon Technology Company. It is a freeze dried power that can be diluted with the supplied vial which contains 30ml of sterile diluent . Each ml of the material contains 200 i.u. of PMSG.

Insertion of the CIDR's :

1)- Ewes were held one by one by a worker in a standing position.

2)- The fat-tail was lifted and the external reproductive genatila cleaned by a wet tissue dipped in a mild iodine solution.

3)- The CIDR was loaded in its applicator and obstetrical lubricant applied to the applicator tip.

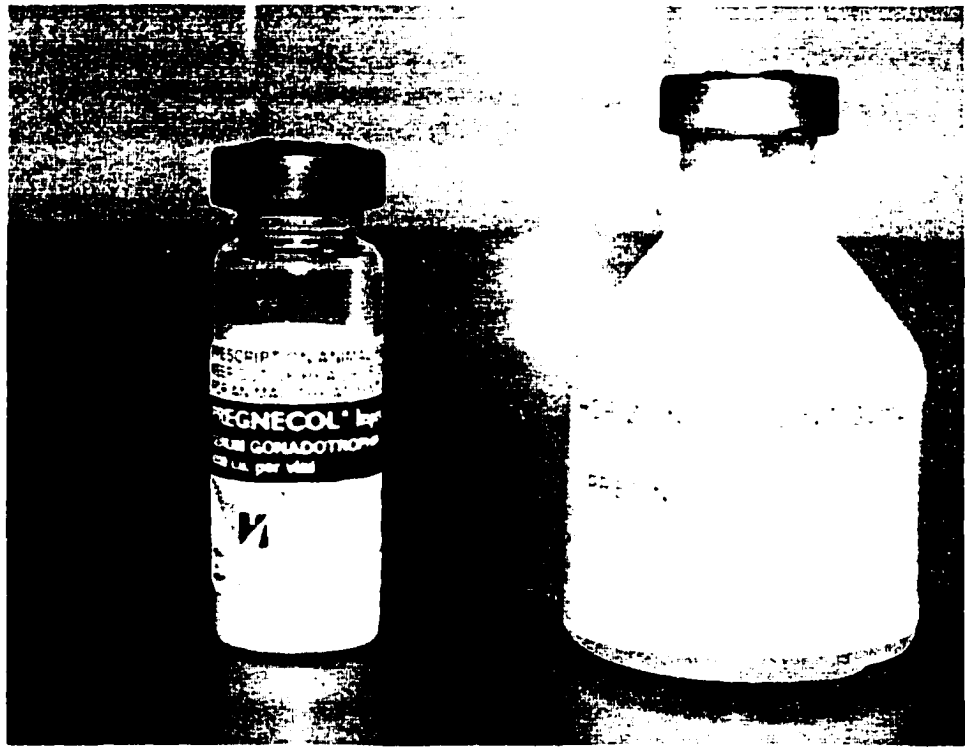
4)- The loaded applicator was gently inserted into the vagina where the CIDR released.

5)- CIDR was left in place for 12 days and withdrawn by pulling the attached string.

Semen Collection and Handling:

The Lane electroejaculator was used to obtain semen from 4 Najdi rams. The semen was collected approximately one hour before the beginning of TCAI on each of three consecutive days. The semen volume, sperm motility were measured. The semen from the four rams was mixed and a sperm concentration was measured using an haemocytometer. Then the semen was extended to $200X10^6$ sperm/ml. using a commercial ram extender (IMV, Minneapolis,MN). After extending, the semen was kept in a test tube at the ambient temperature.

Figure 4: PMSG product (Pregnecol)



temperature (30 ^{o}C) until use. At the time of insemination, the semen was loaded into .5 ml straws.

Sperm Counting :

A Haemocytometer was used for determining sperm concentration of ejaculates:

- The semen was diluted 1:200 with 0.9% sodium chloride contain 0.01% formalin : A pipette designed for red blood cells was used.
- A drop of the diluted semen was allowed to run under the cover glass in one chamber of the haemocytometer. After that, the semen was allowed to settle for approximately 2-3 minutes.
- The haemocytometer was placed onto the stage of a light microscope. First, low power, 10X, was used to determine the large squares of the chamber. Then, the power was increased to 40X for counting.
- The sperm cells in 5 large squares were counted.

Number of Sperm Cell/ml = (Total Sperm Cells in the 5 large squares) X 10 X 10^{6}

Teaser Preparation :

Five healthy ewes, 2-4 years old, were ear implanted with Synovex – H (Figure 5) which contains 200mg testosterone plus 20mg estradiol benzoate. These teasers were implanted two weeks before their use. At the time of introducing these teasers to the synchronized ewes of group 3, they were fitted with marking harnesses to examine efficacy of the treatment.

TCAI Equipment :

The equipments that was used in this study included the following (Figure 6) :

- A vaginoscope (8.5 inches length and 1 inch diameter) with one side opening and an internal sleeve for holding the light source.
- A plunger (10.5 inches length and 0.8 inch diameter) a rod like tube that was used to be inserted into the vaginoscope for easy insertion into the vagina.
- An eleven inches Bozeman forceps to grasp the cervical orifice.
- Light Sources:

  Two different light sources were used :

  1- 10 inch probe lite.

  2- A commercial light source.
- Insemination gun: A half milliliter (ml) Cassou insemination gun which was modified for attachment of a bent insemination tip that is approximately 4 inches in length was used.

TCAI Procedure :

Ewes in group 3 were inserted with CIDR devices in three different days. Thirty four ewes were inserted with CIDR's on day one, 33 ewes in the second day and 33 in the third day, CIDRs were left for 12 days so they were removed on three different days. Therefore, The ewes TCAI'd on three different days. The steps of TCAI that were followed in this trial are:

1- Ewes were placed over a hay bale and held by two workers.

2- The fat-tail was lifted up and the vulva was cleaned with a mild Iodine solution.

Figure 5: Synovex-H

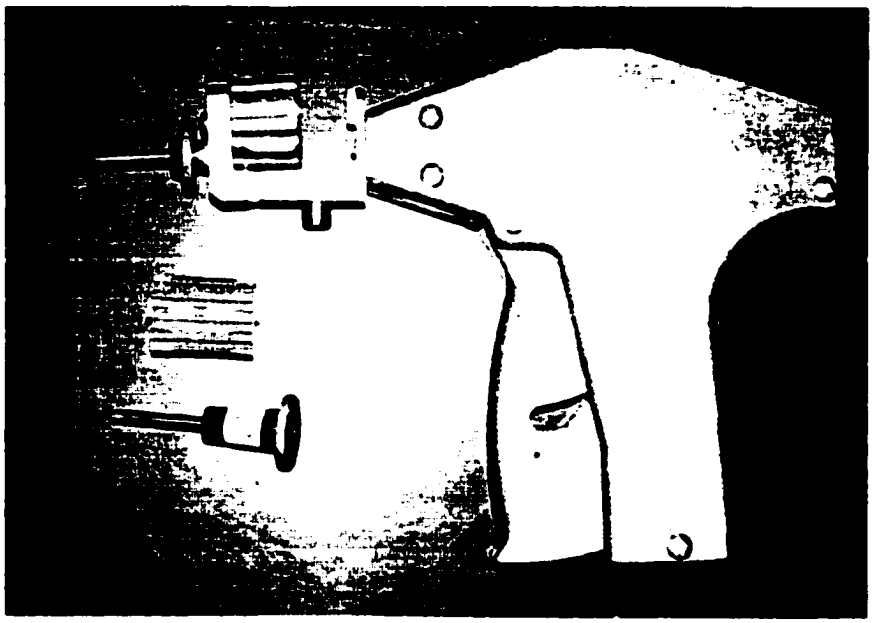
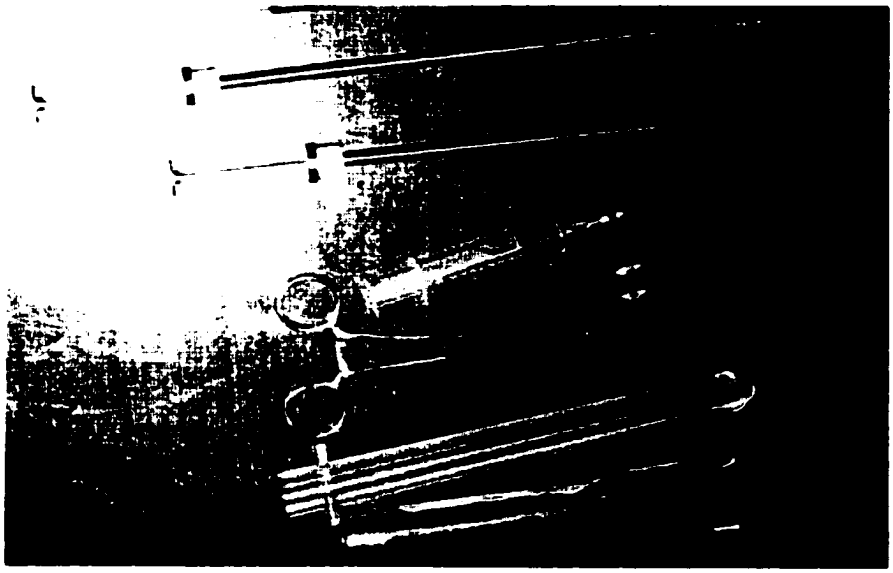


Figure 6: Transcervical AI equipment



3- The plunger was inserted to the speculum and dipped into a cup containing obstetrical lubricant before gently inserting into the vagina.

4- The plunger was removed and the light source inserted into the vaginascope.

5- The cervical opening was located and grasped by the forceps. Any excessive mucus in the vagina was removed.

6- Then, the inseminator gently inserted the tip of the insemination gun through the cervical canal into the uterine lumen.

7- After that, the extended semen was loaded into 0.5 ml straws which in turn were loaded into the insemination gun and semen expelled very slowly.

8- Finally, the vaginascope was removed and a gentle massage applied to the vulvar lips.

Ewes Housing and Feeding :

- Breeding Period : (April 24 – May10th)

Ewes in each group were maintained separately in three different pens but were fed uniformly each day , 500g Alfalfa hay + 250g whole barly grain +250g concentrate (18% crude protein). Water and minerals were fed free choice.

- After breeding : (May 11th – End of May)

All the ewes in the three groups were put together in a large pen and fed the same as during the breeding time. The first pregnancy examination, 20 days post breeding, was performed at the end of this period.

- (June 1st – End of August) :

All the ewes were taken to pasture where they were allowed free choice grazing on aftermath potato and wheat fields. Water provided only once a day and no minerals supplement were given during this period. The ewes were herded back to the facility for the 40 and 60 day pregnancy examinations.

- (September 1st- Lambing) :

Ewes were brought back to a large lambing pen. Where they were fed 600g Alfalfa hay + 300g whole barly grain + 300g concentrate. Water and minerals were available during the 24 hours.

- (Lambing Period) :

After lambing , each ewe was immediately placed with her lamb(s) in a small individual pen. The ewe was fed exactly the same as the last period. Lambing date, type, lamb weight, and gender were recorded.

Time frame:

This study was started in April 22nd and ran through October , 1999.

Statistical analysis:

Chi-square was used to test the difference in lambing rate between the three groups.

Results:

The results of the age and BCS examination of the Najdi ewes in the three different groups are shown in table number 4.

Table:4: ewes ages and BCSs

	Age (years)		B. C. S. (1 – 5)	
Groups	Mean	Range	Mean	Range
Group 1	2.93	1.5 - 5	3.175	2.5 – 4
Group 2	2.85	1.5 - 5	3.245	2.5 - 4
Group 4	2.95	1.5 - 5	3.20	2.5 - 4

Synchronization results :

No CIDRs were lost during this trial.. At the time CIDRs were removed, no abnormal smell nor discharges were observed. The CIDRs of four ewes went deeply into the vagina and had to be removed with a clean forceps. The average ambient maximum temperature was 37°C. The efficacy of treated teaser ewes was tested. They were introduced to ewes of group 3 immediately after the withdrawal of the CIDRs . Several ewes were mounted and marked by these teasers. These treated teasers had developed complete sexual male behavior by the time they were introduced to the ewes.

Semen Collection and Extending :

The age, BCS, and SC measurements of the four rams that semen was obtained from are shown in table # 5 .

Table 5: Age, BCS and SC of the four rams

Rams	Age(years)	B. C. S	SC (cm)
1	3	3	31.5
2	2	3.5	30
3	3	2.5	29.5
4	1.5	3	28

Semen was obtained from these four rams on the three days of collection by applying only one electrical stimulation. The semen volume and color are shown in table #6.

Table 6: Semen volume and color

Rams	Collection 1		Collection 2		Collection 3	
	Volume (ml)	Color	Volume (ml)	Color	Volume (ml)	Color
1	1	Creamy	1.5	Creamy	1.5	Creamy
2	1	Creamy	0.75	Creamy	1	Creamy
3	1	Creamy	1.5	Creamy	1	Creamy
4	1.5	Creamy	0.75	Milky	0.75	Creamy
Total	4.5		5.5		4.25	

The semen dose of $100X10^6$ motile fresh diluted sperm cells was used. The results of sperm cells counting and extending to the final dilution needed in the three inseminating days are demonstrated for day 1 :

Day 1 of insemination:

1- The semen of the four rams were added

1+1+1+1.5 = 4.5 ml

2- The sperm motility of the pooled semen was 70%.

3- The number of sperm cells in 5 large squares of the mixed semen was 150 sperm

Sperm concentration = $150 \times 10 \times 10^6 = 1.5 \times 10^9$

Total number of sperm cells = $4.5 \times 1.5 \times 10^9 = 6.750 \times 10^9$

Number of motile sperm = $6.750 \times 10^9 \times 70/100 = 4.725 \times 10^9$

The total sperm desired in a 0.5 cc straws is 100×10^6

A mount of Extender needed to be added to the 4.5 ml semen

4.725×10^9 = (amount of diluent) $\times 200 \times 10^6$

Amount of diluent = $4.725 \times 10^9 / 200 \times 10^6 = 23.625$ ml

The amount of diluted semen = 4.5 + 23.625 = 28.125 ml

0.5 ml straws were used , therefore each straw would contain (Now, each ml contains 200×10^6 sperm)

0.5 ml straw would contain half this number $200 \times 10^6 / 2 = 100 \times 10^6$

The same steps were followed to obtain the final needed sperm number in the following insemination days.

Artificial Insemination :

In general, the average time of handling and TC insemination of each ewe was 3.5 minutes. The time of inseminating the ewes in day 1,2,and 3 was 2.05, 1.55, and 1.50 hours respectively. The penetration of the cervix was not accomplished in 3 ewes so, the

semen was deposited at the opening of the cervix. None of these non-penetrated ewes became pregnant. Most the ewes had excessive amount of mucus which was taken off before insemination by pulling it out with 20 cm syringe connected to a plastic insemination tube.

Lambing :

There was a significant ($p < 0.05$) difference in percentage ewes lambing and in the number of lambs born per ewe between the three groups. The percentages of ewes lambing in groups 1,2,and 3 were 31, 49,and 53%, respectively. Seven ewes in groups 2 and 3 died during the study, these were necropsied and found to be not pregnant. The average weight of lambs, twins %, triplets % and the percentage of lost lambs are recorded in table #7.

Table 7:

Groups	Group 1	Group 2	Group 3
Average lambs weight	4.3 kg	3.9 kg	4.1kg
Twins %	10	35	42
Triplets %	0	4	0
Neonatal lamb loss % (3 days)	9	11	10

The lambing periods of ewes in group1, 2, and 3 were 22,10, and 9 days, respectively.

Discussion:

It is widely accepted that reproductive performance is the most important economic trait in production. Fields and Sand (88) cited that Willham, 1973, indicated that reproduction in relative economic terms was 10 times as important as production and 20 times as important as products. However, the response to reproductive selection is very low, 10%, with reproduction when compared with production, 40%, and products 50%. In sheep, in addition to the low hertability of reproductive traits, seasonality plays a major role on their reproductive performance.

At NADEC sheep farm, the optimum goal is to have more than one lamb crop each year. However, the change in day length which is associated with an increase of ambient temperature from the beginning of May until the end of September decreases the ability to achieving this goal. In addition, Najdi breed has a fat-tail which can also add to this problem.

Docking their tails is a good management practice which can be done and thereby eliminates the problem of the fat-tail which can be a barrier to completion of natural mating. Shelton, 1990, examined the effect of docking of fat-tailed of Karakul ewes on their reproductive performance (89). He found that the lambing percentage was significantly higher for docked ewes, 92.9%, on comparison with undocked ewes, 78.9%. Docking fat-tailed Najdi sheep is not done because consumers do not accept Najdi lamb without the fat-tail. In my opinion, docking of females that will be kept for breeding is a management practice that can result in improved reproductive performance.

Estrus synchronization and artificial insemination are two additional methods that can enhance sheep reproduction. In this study, sheep CIDR's were successfully used to synchronize estrus in Najdi ewes. No complications were encountered with either inserting or withdrawing them. No CIDR's were lost from the ewes during the treatment period. At least part of no CIDRs being lost may be due to the ewe's fat-tail which covers the external reproductive tract keeping the CIDRs secure.

In general, the average reproductive performance of Najdi sheep, at this farm, is low with a lambing rate of less than 50% over the last three years. This study resulted in satisfactory lambing rates and percentages of multiple births in treated ewes. In the control ewes, the recent weaning and introduction of eight rams may have contributed to the 31% lambing rate. However, PMSG injections in treatment groups enhanced the ovulation rates resulting in higher percentages of multiple births.

The average daily ambient temperature prior to, during and after the breeding dates was approximately 35 C. This high temperature may have affected fertilization rates and the survival of the embryos during their early development. It was found that fertilization and early embryonic stages are the most sensitive periods of high ambient temperature (10). Two abnormalities were detected in the 181 born lambs and this was considered normal for NADEC. This study also revealed that good ejaculates can be collected from Najdi rams even when ambient temperatures are relatively high. The male to female ratio in this farm is very high. They normally use an average of 8 rams for 100 ewes and in some pens more than 10 rams per 100 ewes are used. In the control group, 8 rams per 100 ewes were used which is still excessive. The rams were seen spending most of their time fighting with each other and preventing each other from breeding ewes

in estrus rather than actually breeding ewes. In the second group, 10 rams per 100 ewes were used to breed the synchronized ewes. The reason for using this higher ratio is because the ewes come into heat together in very short period. Some authors suggest using 20 rams per 100 ewes when ewes are synchronized during their non-breeding season because the rams' libido is low during this time. At the time of doing this trial, the Najdi rams had not yet lost their reproduction activity.

The use of transcervical insemination gave good results. The cervical penetration rate was very high (97%) and only 3 ewes were not penetrated. The high penetration was likely due to the fact that these ewes had recently lambed. The average age of the ewes was approximately 3 years, and many had likely lambed more than once which increased the overall size of the reproductive tract. Holding the ewes in a standing position may have also contributed to these results. Finally, it was stated that there may be anatomical variations among the sheep breeds in regard to the cervical anatomy (90) and Najdi sheep have a more "goat like" cervix.

Teasers can be prepared in a number of ways (6). In this study treating healthy culled ewes with male hormones made them act like males. Implanting ewes with Synovex-H is easily performed and produced good results. Introduction of treated teasers to the ewes at the time CIDRs were removed but before insemination improved the synchrony of ovulation in the ewes and helped ensure ewes are in heat (64).

It is likely that the majority of lambs that died in this study was due to the very hot temperatures. The average maximum temperature during the lambing period (September 15- October 10^{th}) was $40^{o}C$. Most the lambs (85%) died in their first day. These were weak lambs and died due to respiratory problems. Providing more

environmentally suitable lambing pen could help decrease the mortality rate of new lambs born at this time of the year.

In my opinion, there are several things that need to be considered at NADEC Farm to raise the performance of Najdi sheep:

1- The nutritional status of the animals is below the required level. For example, the amount of dry matter provided to the ewes during the last month of gestation, 1.2kg (2.64lb), covers only 70% of their needs. Therefore, many weak animals were observed. Increasing the nutrition level would be very important for these animals to maximize reproductive capability.

2- The general health of the animals in this farm were not ideal. There were many animals with mange, abscesses, and low body weight. These problems should be eliminated and eliminating the health problems would enhance the productivity of Najdi sheep in this farm.

3- It is very important to introduce new genetics to this flock through purchasing new rams. On this farm, no rams from outside farms have been used during the last six years and several genetic problems have started to appear.

4- Crossing Najdi sheep with other breeds that have high productive performance can result in having crossbred animals that are accepted to the consumer and at the same time have higher productivity and still have the ability to thrive in the local environment. The results that have been reported in this chapter indicate that using CIDR-G devices for synchronizing Najdi ewes during summer months gives satisfactory results. PMSG use increased the twinning rate as well as using trans-cervical insemination in conjunction with CIDR also gave reasonable results.

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CHAPTER 3
PREGNANCY EXAMINATION

Introduction:

Early diagnosis of pregnancy can be of considerable benefit to sheep producers. Several methods for pregnancy diagnosis in sheep have been used. However, the cost, accuracy , safety, and delayed results have limited their use in practice. Traditionally, shepherds have diagnosed pregnancy in their sheep by using very simple methods. Non return to estrus, abdominal palpation, abdominal enlargement, and udder development are the most common traditional methods that have been used . In recent years, the use of real-time ultrasonic technique has proven its usefulness to sheep breeders. Real-time ultrasound technique can be applied at the farm and gives early and accurate results. In addition, this technique allows for determination of fetal numbers, which can allow for greater management options.

At NADEC sheep unit, the ultrasonic diagnosis of pregnancy in Najdi sheep is often done at three months post breeding. The reason for diagnosing pregnancy at this time is to achieve higher rate of pregnancy detection. One goal of this present study was to examine the possibility of getting high diagnostic accuracy when ultrasonic technique was used for pregnancy testing at three early periods, 20, 40 and 60 days post breeding.

Literature Review:

Knowing whether an animal is pregnant or not is of considerable economic value. Early diagnosis of pregnancy has economic value to the sheep industry and to producers because accurate techniques for early detection of

pregnancy are valuable for improving economic, nutritional, reproductive management (1, 2).

Advantages of pregnancy diagnosis:

1)- Economic:

The economic advantage of using pregnancy diagnosis includes the following points:

a) Farmers can cull or rebred non-pregnant ewes thereby reducing feed expenses and the number of ewes that don't produce lambs (3, 4).

b) Decrease the cost of pre-lambing drench and vaccination procedures.

c) No need for crutching open ewes.

d) Keeping only pregnant ewes at lambing paddocks will decrease the cost and the time of watching ewes at lambing time.

e) Non pregnant ewe lambs can be sorted off and managed differently or culled and sold as fat lambs. This will facilitate better use of feed, facilities and labor (5).

f) Diagnosing the ewes before slaughtering them is of considerable economic importance because it can prevent slaughter of pregnant ewes.

2)- Management and nutrition:

a) Producers can re-breed open ewes during the cycling period (6)

b) Identification of ewes carrying more than one fetus gives the producers the chance to apply good nutritional management based in fetal numbers during late gestation.

c) Feeding ewes carrying multiple fetuses according to their nutritional requirements helps to prevent pregnancy toxemia (7).

d) The ability to determine the age of fetuses gives the producer the approximate time of lambing so they can plan for the coming lambing season in terms of facilities, labor, feed needed and grafting strategies.

e) Detection of numbers of fetuses could offer earlier treatment for various disorders at lambing (8).

f) Well nourished twin-bearing ewes produce more milk, which positively affects lambing and weaning weights.

g) Reduce losses from dystocia by avoiding overfeeding of single fetus bearing ewes (5, 9, 10).

h) The determination alloes grouping ewes according to stage of gestation and number of fetuses being carried could improve lambing management (5, 11).

I) Dairy sheep owners especially want to get information about the pregnancy status of their animals early so that plans for subsequent breeding can be made.

j) Purchasing tested ewes from an auction barn gives buyers confidence about the status of what they have just bought (12).

k) Early determination of embryo numbers allows evaluation of whether or not certain management regimens improve embryonic and (or) fetal survival (13).

l) The determination of fetal sex gives the producer an idea about the number of female and male lambs they will get at lambing.

3)- Reproductive:

a) Pregnancy testing can help in the diagnosis of reproductive problems.

b) Pregnancy detecting enables the producer to direct diagnostic efforts towards either failure of conception or lamb mortality (14).

c) The producers can early detect any reproductive disorders in their ewes and provide suitable treatments (14).

d) Some of the pregnancy diagnosis methods can give information about the sex of the fetus and this is very useful especially to those scientists who are trying to separate sperm carrying Y or X chromosomes and use those sexed sperm in artificial insemination(AI) programs. They can thus determine the accuracy of their methods at an early stage.

e) The ability to predict pregnancy early in ewes with a high degree of accuracy provides an excellent opportunity to study embryonic and fetal development(13).

5)- Other reasons :

a) Maidens that fail to lamb may be less likely to lamb as adult ewes(14). Therefore pregnancy testing could be used as part of the selection procedure for maiden ewes.

b) For selection programs, the determination of the number of fetuses is fundamental to those breeders who want to retain adult ewes bearing twins for subsequent selection of ewe lambs for replacement in the ewe flock(11).

c) When veterinarians perform pregnancy diagnosis, they will discuss flock health programs with producers who will benefit from the consultation and recommendations.

c) Pregnancy testing can provide a cost effective service to producers and income for veterinarians and their agents that offer this service.

c) For the research physiologist, using some of the pregnancy diagnosis methods, such as vaginal biopsy, might provide a guide to the action of hormones on the reproductive tract(15).

THE METHODS OF PREGNANCY DIAGNOSIS IN SHEEP:

There are many methods for pregnancy diagnosis in sheep. Some methods give a higher degree of accuracy at the early stages of gestation. Some of them can give an indication of fetus numbers and others can indicate the age of the fetus. Many pregnancy diagnosis techniques have been used in sheep prior to the advent of ultrasound techniques.

1)- Non-return to estrus:

Estrous cycle is the period of time from the beginning of one estrus to the beginning of the next estrus in the female. Sheep are seasonally polyestrus animals. The length of estrous cycle in sheep ranges from 14 to 19 days with an average of 16-17 days. Estrus cycle can be divided into four stages :

a- Estrus period : which is the period of sexual receptivity where the ewes show estrus behavior such as standing to be mounted by rams, and seeking out rams. This period will range from 24-36 hours. The first day of estrus is day zero of the cycle. If the female is not pregnant, it will show estrus behavior every 16 -17 days.

b- Metestrus : time when corpus luteum (CL) is developing

c- Diestrus : period of cycle when the CL is mature.

d- Proestrus : the period when the CL begins to regress and continues until the beginning of the next estrus period.

e- Anestrus : period of time when female is not in estrus during certain physiologic states, e.g. before puberty, during pregnancy and lactation, and in seasonal breeders. Some breeds of sheep show seasonal anestrus due to the changes of photoperiod, which affect the secretion of GnRH from the hypothalamus and FSH and LH from the anterior pituitary. As a result, the ovaries become small and do not contain follicles or corpora lutea. Also ,the serum concentration of LH, progesterone, and estriadiol are low.

During metestrus, diestrus and proestrus, no estrus behavior can be seen in ewes. If a ewe doesn't show estrus behavior after 16-17 days from the last mating, this could be taken as an indication of pregnancy. However, the sign of non-return to estrus due to pregnancy is not physically different from seasonal anestrus at the end of season in sheep that exhibit seasonality in estrus behavior.

Rams or vasectomized rams, fitted with a harness with an attached colored crayon can be used to determine non-pregnant ewes that are in estrus (16). The accuracy of determining non-pregnant ewes by this method is dependent on the number of cycling ewes and this in turn depends on the season and the body condition score of the ewes. When marked ewes are put with fertile rams for period of time, they will not be marked again by a harnessed rams if they became pregnant. Richardson (3) cited that Radford and Wood used this method and they found that around 95% of pregnant

ewes were marked at mating and four per cent of those pregnant ewes were not marked at this time and one per cent of those ewes were marked but they did not lamb until after 156 days. Watt (3) cited that Knight et al. did two experiments examining the accuracy of pregnancy detection by using harnessed rams. In the first study, 1975, they investigated this technique on 3704 ewes joined from late October to early March. They obtained 63.7 % and 97 % accuracy in determining pregnant and non-pregnant ewes, respectively. In their second study, 1979, they obtained higher accuracy of determining pregnant , approximately 99% and open ewes, approximatly 70%. This method is very simple to use, has very low cost, and allows for early removal of unmarked ewes from the rest of the flock. However , this method isn't very accurate when used out of season where there are many ewes that don't show estrus even when they are not pregnant and will not be marked by harnessed rams.

2) Abdominal palpation:

This is a common method that has been used by sheep owners. The shepherd can do this method by keeping the ewe in standing position and lifting the abdomen frequently in front of the udder and trying to feel the fetus with their palpating hands(17). The accuracy of pregnancy diagnosis using this method is acceptable if it is done after 80 days of gestation. Prott et al. obtained 80 to 95 percent accuracy when they diagnosed ewes at 90 to 130 days of gestation.(17). The body condition of the ewes can affect the accuracy of this method. Holding feed and water for 12-17 hours makes the diagnosis much easier, especially for

over weight ewes. Abdominal palpation is easy to perform and does not require special equipment. Experienced operators can diagnose around 200 ewes per hour if they have sufficient assistants catching and holding the sheep(17). Even though it is an inexpensive method that can be used in sheep, it is not very accurate and does not give good results over the early periods of gestation.

3) Rectal abdominal palpation:

This technique was perfected at the U.S. Sheep Experiment Station at Dubois, Idaho, for pregnancy detection in sheep(18), and has been repeatedly used for years in undeveloped countries. This technique has also been used in goats(19). This procedure can be done very easily by applying the following steps :

1- the ewes should be held off feed over night to facilitate the procedure.

2- the ewes are placed on a laporatomy cradle for examination.

3- Six to eight oz of soapy solution is injected gently into the rectum with a drenching gun.

4- A hollow plastic rod 1.5 × 50 cm. with a rounded tip is lubricated and inserted gently into the rectum to a depth of 30-35 cm.

5) The operator uses one of his/her hands to manipulate the plastic rod and moved up and down and from side to side until the operator feel that there is something in the uterus. Then, the uterus can be lifted up toward the abdomen where the operator uses the other hand to feel if there is a fetus in the uterus. This method is about 97% accurate at 60 days post mating and requires 30 seconds per ewe. In general, this rectal-abdominal palpation technique is simple, quick and

inexpensive. However, rectal trauma, abortion and death have been reported(20,21,22,23).

4) External appearance:

A farmer can use this method to distinguish advanced pregnancy by looking to those ewes with larger than normal abdomen. It is useful method that can be adjusted to other pregnancy diagnostic techniques. The large abdomen of pregnant ewes is evident at late stage of pregnancy. This method does not give good results when the ewes are very fat and during the early stage of pregnancy .

5) Mammary development and its secretion:

The size of the udder can be used for pregnancy diagnosis especially when when dealing with ewe lambs that are pregnant for the first time. The development of the udder is commenced in some ewes as early as 71 to 90 days of gestation. By 111 days to 130 days, 96% of ewes have at least moderate udder development and after 130 days 84% of ewes have obviously enlarged udders (24). However, there are some ewes that do not develop a large udder by the end of gestation.

The nature of the udder secretion can be used as an indicator of the pregnancy. Richardson (3) cited that Hokler (1962) mentioned that a sticky udder secretion was 100% accurate in indicating pregnancy in ewes after 51 days of gestation . In another study, Ishwar (19) cited that Webb found pregnant ewe lambs produced a sticky honey-like mammary secretion 2 months before lambing. Richardson, however, did not observe this honey-like secretion when he

tested 11 ewes at 30-80 days of gestation (3). This method is not accurate and at the best gives late results.

6) Increase in body weight:

The body weight of a pregnant ewe partially increases during pregnancy due to increased secretion of some specific hormones, such as progesterone, estrogen and growth hormone, which increases efficiency of using the feed by pregnant ewes. Most of the body weight gain happens during the last trimester of pregnancy due to the rapid growth of the fetus. Ewes carrying single lambs had a gain of 6-12 percent while ewes carrying twins have around 13-16% gain of their body weight(25). The increase in body weight is not an accurate method for pregnancy diagnosis in sheep however(3).

7-Vaginal Biopsy:

Much work has been done to determine the histological changes in the reproductive organs of the ewe during anestrus, the estrus cycle and pregnancy (26). Richardson used the ovine vaginal biopsy for pregnancy diagnosis where small pieces of vaginal mucosa were removed from living animals by using a biopsy instrument and the sample was subjected to routine histological procedures (15). A histological slide of the mucosa was examined under a microscope where they found that there were differences between the number of vaginal epithelial cell layers between the non-pregnant and pregnant ewes. The vaginal epithelium of non-pregnant ewes was composed of 12 cell layers while only five layers were present from the first month of gestation until 20 days before the lambing in pregnant ewes. Also, the luminal cells of the vaginal epithelium of open ewes

were flattened squamous with deep staining nuclei and the deeper cells were polygonal with bright staining spherical nuclei in which the chromatin structure was clear. On the other hand, pregnant ewes had columnar, cuboidal and prismodial cells. In 1972, Richardson obtained 100 % accuracy in determining pregnancy by using this method after 80 days of pregnancy and 97 % after 40 days (3). This author reported around 81% accuracy of diagnosing non-pregnant ewes (15). As such this technique is relatively inaccurate as well as impractical.

8- Palpation of uterus and ovaries via laparotomy:

The uterus and ovaries can be palpated directly through the abdomen wall. The operator makes a small incision in front of the udder to allow 2-3 fingers to go through that opening. Then, the uterus and the ovaries can be palpated and the operator can decide if a ewe is pregnant or not. Open ewes have a small uterus and there is no corpus luteum on the ovaries. At 4-5 weeks of pregnancy , the uterine horns will appear distended. After six weeks of gestation, the cotyledons can be palpated. In general, an enlarged thin walled uterus containing fluid is taken as positive evidence of pregnancy. Aseptic technique is necessary during laparotomy to prevent infection. Lamond (1963) obtained 97% diagnostic accuracy when he applied this technique on 278 ewes at 4-8.5 weeks post breeding (27). He examined 24-27 ewes per hour when he used only one assistant. Five years later, Hult and Foote applied this technique and obtained 92% accuracy in diagnosing pregnancy in ewes that were 4-5 weeks pregnant (28). In 1972, Hult used this technique for diagnosing multiple pregnancies, and he obtained 90,92, 92 and 50% diagnostic accuracy in ewes carrying 0, 1, 2 and 3 fetuses ,

respectively (29). The ewes were around 22-30 days of pregnancy. Ishwar (19) cited that Smith obtained 100% accuracy when he applied this method on a group of ewes that were more than 42 days pregnant. This method is accurate but it can not be done in the field as a commercial way for pregnancy diagnosis in sheep due to practical and animal welfare constraints.

9- Laparoscopy technique:

This is another technique, which cannot be used commercially under field conditions because this procedure must be done in a lab under very sterile conditions. The procedure can be done by inserting a laparoscope into the abdomen for visualization of the uterus and ovarian structure(14).

Determining an early pregnancy can be made after 17 days of gestation by observing the presence of the (CL). After 25 days of gestation, uterine horn enlargement can be taken as an indication of early pregnancy (14). Phillippo et al obtained 93.3% diagnostic accuracy of pregnancy after 17 days of gestation (30). this study, also used this technique to determine the number of fetuses present by counting the number of corpora lutea.

10- Palpation of the cervix:

This technique involves the palpation of the external cervical os through the vagina at 50 days or more post breeding. A very soft, blunted cervix or the inability to reach the cervix can be taken as an indicator of pregnancy while a firm conical-shaped cervix projecting into the vagina is suggestive of non pregnancy (19). However, an enlarged uterus filled with pus(pyometra) would give the examiner the impression of pregnancy.

11- Blood metabolites and Hormones:

Various attempts have been made over the years to use some circulating hormones, metabolites, and pregnancy specific proteins for diagnosing pregnancy in sheep.

A- **Hormone concentration:**

Measurement of some steroid hormones concentration such as progesterone and estrogen at specific times post-breeding has been proven to be an accurate method of pregnancy diagnosis in farm animals (31, 32, 33, 34, 35, 36). Sensitive radioimmunoassays and enzyme immunoassays are available for the measurement of the concentration of these hormones.

1- Progesterone test:

Measurement of the concentration of progesterone in blood and milk has been used for many years for diagnosing pregnancy in many species of animals. During the season and following ovulation, the progesterone concentration in the blood of cycling ewes increases up to the 10^{th} day and remains at higher level until the 14^{th} day. By day 14, if there is no conception, the uterus secretes high amounts of prostaglandin hormone that destroys the CL (s) and this results in a sharp decline of progesterone levels in the blood. Thimonier et al. (1977) measured the progesterone concentration in the plasma after 18 days of mating(37). They showed that all females diagnosed as non- pregnant did not lamb and 85.5% of those diagnosed pregnant did lamb . In their study, Lohi ewes and Lohi × Awassi crossbred ewes were used for determination of progesterone concentration

using enzyme immune-assay techniques in blood plasma for early pregnancy diagnosis. Blood samples were collected on alternate days from onset of estrus till day 22 and at days 32 and 34 of gestation. It was concluded that a progesterone level of 3.10 ng/ml or above after 16 days could be taken as an indication of early pregnancy in these breeds . Brundige et al. (38) measured serum progesterone concentration of 25 female Bighorn sheep (*Ovis canadensis*) in February 1984 and January 1985. They concluded that obtaining 2 ng progesterone or more per milliliter of serum could be taken as a marker of the pregnancy. They achieved 90% and 80% accuracy in 1984 and 1985, respectively .

Progesterone concentration may be also taken as an indication of multiple pregnancy. Bassett (1969) reported that progesterone concentration during the last 50 days of gestation in ewes with twins was twice that of ewes bearing singles (39). Gadsby et al. tried to diagnose multiple pregnancy by using the concentration of progesterone in the blood and obtained 69% diagnostic accuracy (40). More recently, Schneider and Hallford collected 341 blood samples, at 100 days of gestation, from ewes between one to seven years old. In this study, ewes with progesterone $\leq$ 2.4 ng/ml were considered non-pregnant , while ewes with progesterone concentration $\geq$ 2.5 ng/ml were considered pregnant. Ewes with progesterone from 2.5 to 10.9 mg/ml had one lamb and ewes with more than 11 ng/ml had multiple lambs. They obtained 98% accuracy in diagnosing fetal numbers.

2- Estrogen assays:

The estrogen concentration in the blood increases during the last period of pregnancy due to the increase of placental secretion of this hormone. Drane et al. estimated estrogenic content, estrone and B-estradiol, of 38 plasma samples which were obtained from six pregnant ewes (41). The result of those samples indicated a rise in bound estrone activity in the magnitude of 2.5 ng estrone per 100 ml plasma to 20 ng/100ml plasma during the last six weeks of pregnancy . Thimonier et al. (1977) estimated the total estrogens between 100-110 days of gestation (42). They obtained 98% diagnostic accuracy in pregnant ewes and 94% of non-pregnant ewes . In another study, a total of 105 primiparous and multiparous ewes were subjected to pregnancy diagnosis by measuring the blood estrogen level between 38 and 81 days of gestation (46). The accuracy of diagnosing pregnancy by using this method was 90%. Fecal estrogen content can be used for pregnancy diagnosis but this could be affected by the amount of estrogen components present in the diet. Bamberg et al. mentioned that fecal estrogen can be used for pregnancy diagnosis in horses, cows but not in sheep, pigs, and goats (43).

Pregnancy- specific protein B:

Pregnancy specific protein B (PSPB) was first detected in the bovine placenta in 1982 and was isolated from ovine placenta in 1988 (44) . The binucleate cells of the fetal trophectoderm secrete this protein. It can be detected in the blood of sheep as early as 20 days after mating. There is a positive relationship between the concentration of this protein and the number of fetuses

carried by the ewe. Therefore, this method can be used for diagnosing single vs. multiple pregnancies. This test was 78% accurate in distinguishing twin from single pregnancies between days 60-120 (45). The concentration of PSPB in the blood is affected by many factors, such as the time of gestation, the size or age of the ewe, the breed of the ewe and the breed of the fetus.

B- Blood Metabolites :

The concentration of some blood metabolites such as glucose, non-esterified fatty acids and ketone bodies have been taken as an indication of pregnant or non-pregnant ewes and to determine if the ewes carry single or multiple fetuses (3, 46, 47). Blood glucose levels decline during pregnancy especially in ewes bearing multiples and measuring this decline gives late results with a reasonable level of diagnostic accuracy.

12- Radiography:

Radiography can be used for determining pregnancy in small ruminants (53). There have been many attempts to use radiographic technique to detect pregnancy and fetal age (48, 49, 50). This technique is based on the identification of the fetal skeleton on an x-ray plate. This technique can be used also to determine multiple birth with 90% accuracy or more if made after 90 days of gestation (51). This method can be 100% accurate when it is used to determine pregnancy in smaller breeds of ewes using one film after 71 days post-breeding (51). The fetal skeleton is often radiolucent by 65 days of gestation. However, the change of uterus size can be detected earlier than 65 days of gestation but may

not be differentiated from some uterine diseases such as hydrometra and pyometra (19).

There are three types of radiographic units that have been used (Watt, 1983)(14) :

1- Portable diagnostic units:

These small portable x-ray units can be used for diagnosing pregnancy from 60 to70 days of gestation with up to 96% accuracy. This method can not be used commercially because of time constraints , potential danger to the operators, and the cost of individual films.

2- Modified field unit:

This unit is able to examine ewes at 50-75 days with an accuracy of around 95%. However, this method gives its best results when ewes are examined at least 100-120 days. This type of radiography , while likely safer, also can't be used commercially because it is very expensive, and results are delayed.

3- Video Fluoroscopy units:

The first use of this method for diagnosing pregnancy in general and multiple fetuses was in New Zealand in 1982 (52). Ewes are restrained in the standing position for fluoroscopy and then are tested at 90 days or later in gestation. Two operators are required for accomplishing this method. One hundred ewes can be examined per hour. The accuracy of using this technique is 100% for determining single pregnancies and approximately 95% for separating single versus multiple pregnancies.

Using radiography for pregnancy diagnosis is very accurate but it has some disadvantages, which include:

- Does not give an early result of the pregnancy.
- A costly method.
- Causes a radiation hazard to the operator.
- Requires the animal to be restrained.
- Not practical for examining large number of ewes in the field.

13- Ultrasonic Techniques :

Ultrasound technology has been the subject of considerable interest for the last 87 years when Richardson (1912) invented a sound wave device to warn ships at sea from large objects in the water (55). Ultrasonography was first used in human medicine in 1942 by Dusik (56). Subsequently, this technique was used widely to detect gallstones and foreign bodies in soft tissues (57). The first use of ultrasound technology for diagnosing pregnancy in humans was by Donald et al. in 1958 (58). Ultrasonic technique was first used in cattle in 1962 for estimating rib eye area and fat thickness (59). In 1966 this technology was first used in diagnosing pregnancy in sheep (60). More recently, this method has been used to diagnose multiple fetuses, the fetal age, sex, and developmental abnormalities in sheep and other species.

Ultrasound is nothing more than sound at a frequency that can't be detected by the human ear (61). Humans can hear sounds in the range of 20 to 20,000 hertz (Hz). That frequency is approximately 20,000 cycles per second or 20 kilohertz (kHz). Diagnostic ultrasound uses frequencies in the range of 2 to 10 million cycles per second or 2 to 10 megahertz (MHz). In therapeutic applications, ultrasound frequencies are much higher than those used in diagnostic settings . Ultrasound at 2 to 10 megahertz is produced by transducers containing piezoelectric crystals, which can transfer electrical energy into

mechanical energy as well as the opposite. The transducer or the probe is that part of the ultrasonic system that is used in contact with the animal's skin or rectum. The usual transducer frequencies used for pregnancy diagnosis range from 3.5-7.5 MHz. The lower frequencies transducers will penetrate much deeper than the high frequency probes, however lower frequency probes have poorer resolution.

Two types of ultrasound are used in human and veterinary medicine :

1- Doppler ultrasonography:

The principle involved in Doppler ultrasonography for the diagnosis of pregnancy is to detect vibrating membranes like fetal heart beat, fetal movement and umbilical blood flow (62). When the ultrasonic waves contact a moving target, the frequency of returning echoes is different from the sender signal. This principle can be used to diagnose pregnancy in sheep. Either trans-abdominal or trans-rectal transducers can be used. A trans-abdominal Doppler transducer is used with the ewe restrained in the standing position. Hair or wool in front of the udder is clipped and some lubricant agent such as commercial ultrasonic gel or vegetable oil is applied to the clipped area to provide good contact with skin. The transducer is placed on the ventrolateral abdominal wall and the operator listens for the fetal heart beat, which is a rapid clapping sound or the swishing sound, which represents the umbilical artery blood flow. In addition, the movement of the fetus and the sound of the uterine arteries can be detected by this machine. The rectal Doppler probe is used with the ewe held and restrained in a standing position with the operator standing behind the ewe. In this case, the lubricated rectal probe is inserted into the rectum. The operator listens to the sound of uterine arterial blood flow which can be heard when the probe is inserted 5-10 cm into the rectum (14).

The intra-rectal Doppler technique is better than external technique when used at the beginning of the second trimester (19).

Pregnancy Diagnosis can be conducted as early as 45 days but is more accurate if done after 60 days (63). Many trials have been done to evaluate the accuracy of using Doppler ultrasonic devices. Callagon et al. (1964) were the first people who introduced the Doppler ultrasonic method for diagnosing pregnancy in humans (64). In (1967) Fraser and Robertson were the first to apply this technique for pregnancy diagnosis in sheep (65). Shone and Fricker (1969) obtained 100% accuracy in diagnosing 309 ewes between 66 to 122 days of gestation (66). Thwaites (1981) reviewed most the trials using trans-abdominal Doppler devices (67). In general, the accuracy of pregnancy diagnoses is over 90% when ewes are diagnosed 80 days after the breeding (68, 69, 70).

There are many experiments that have been done for evaluating external Doppler ultrasonic machine. Lindahl reported that rectal devices could be used as early as 25-30 days post breeding and found greater accuracy if the diagnosis is between 35 to 40 days (71). Deas obtained over 90% accuracy of diagnosing pregnant ewes with rectal devices after day 40 of gestation (72). Watt et al. (14) achieved over 80% accuracy when they diagnosed pregnancy after day 70. Watt pointed out the main problems of using the Doppler device; is that they are slower than other ultrasonic machines such as echo pulse instruments and that very poor contact may occur with the internal instrument.

Diagnosing multiple pregnancy by using Doppler devices is not very accurate . The operator tries to detect heart beat of each fetus. Richardson (3) and Fukui(8) et al. tried to diagnose multiple pregnancy but reported disappointing results. However, Fraser

et al. (1971) reported encouraging result when using the external device and obtained an 82% accuracy in diagnosis of fetal numbers(73).

2-Pulse-Echo ultrasonography :

This method uses pulses of the ultrasound generated by piezoelectric crystals in a transducer. When the pulses contact tissues, they are reflected back to the transducer and converted to electrical energies that are displayed on either A or B mode.

A-mode ultrasonic technique (echo amplitude):

The principle of the A-mode ultrasonic technique for diagnosing pregnancy is by detection of a fluid-filled uterus (14). A-mode is simpler than B-mode . The origin and the amplitude of the A-mode echo are displayed as spikes originating from a vertical baseline. There are many commercial A-mode detectors such as scanopreg ®, pregnosticator ® and pregmatic 3 ® detectors. The transducer is placed on the lower right flank in front of the udder of the standing ewe. Vegetable oil has been used to provide a good contact between a transducer and the skin. The best result can be obtained when the pregnancy examination is done between 60-120 days of pregnancy(14). O'Halloran (1980) compared two types of A-mode ultrasonic detectors, scanoprobe and scanopreg (74); he found that using scanoprobe gave 11, 56, 83, and 49 % accuracy when it was used on days 40, 60, 80, and 110 respectively. However, he obtained much better results when he used the scanopreg device; 20,69,86,96 percentage diagnosis on days 40, 60, 80, and 110 respectively (3). Watt (14) cited that Lane and Lewis obtained ninety eight percentage accuracy of pregnancy diagnosis by this method. Haibel(1990) mentioned that at least 95% diagnostic accuracy can be obtained when A-

mode scanners are used between 60-80 days (10). An experienced operator with good facilities and assistance can examine approximately one hundred ewes per hour. A-mode devices are not very accurate for diagnosing single vs. multiple fetuses.

B-mode technique :

B-mode ultrasonic imaging is a two dimensional display of dots on a cathode ray tube (CRT) screen (19). In this case the loudness of the echo is displayed as bright spots on CRT screen. The "B" in B-mode refers to the brightness of the spots that can be displayed in different levels, which are called the gray scale displays. Tissue characteristics determine what percentage of the sound wave will be reflected. The reflected portion is represented on the CRT screen by shades of gray extending from black to white. Liquids such as embryonic fluids do not reflect sound waves, thus black appears on the CRT screen. On the other hand , bones reflect much of the sound waves, therefore they will appear as a white on the CRT screen. Other tissues can be seen as different shades of gray and this depends on the reflection degree of the ultrasound waves. B-mode ultrasonic scanners produce a frozen sectional picture of the tissue being scanned. Lindahl tested a group of pregnant ewes with the scanogram, which is an example of the B-mode devices, and obtained 100% diagnostic accuracy of pregnancy and 65-85% accuracy of diagnosing multiple fetuses when he diagnosed those ewes in the last half of pregnancy(75).

Real-time B-mode ultrasonography:

Real-time refers to the ability to see a live or moving display on the ultrasound image (61). Images on the cathode ray tube screen are composed of frames, so the image, which is displayed, appears continuous, thus producing real-time images. The

images change due to the movement of the transducer or structures. Real-time B-mode scanners display a moving gray-scale picture of the cross-section of the tissues examined. The high density tissues like bone will be displayed as a bright structure while a liquid or gas will be displayed in CRT screen as dark areas (61). Other tissues such as muscle, uterus and placentomes can reflect part of the ultrasound beams and will be displayed as gray areas.

B-mode real-time ultrasound was developed in Australia in 1970s for the diagnosis of pregnancy and fetal abnormality in humans (76). Approximately ten years later, the technique was used for pregnancy diagnosis in the horse offering an early diagnosis (76). Wittman et al. used real-time ultrasound to study the breathing and body movement of the full-term sheep fetus(77). The use of real-time ultrasound scanners for detecting pregnancy in ewes was first done in 1982 at an experimental station in Australia by Fowler and Wilkins (78). They obtained 100% accuracy in diagnosing pregnancy from 40 days of gestation.

Real-time scanners:

There are two types of real-time B-mode ultrasound scanners available for pregnancy diagnosis(79). These scanners are categorized by arrangement of the piezoelectric crystals and by the method of electrical stimulation of those crystals, into linear-array or sector scanners.

The linear-array scanners have series of 64-120 crystals placed next to each other and arranged in a line, which is usually 5 to 10 centimeters long. These crystals are electronically fired in sequence. Each crystal produces a sound wave that travels parallel to waves that are produced by neighboring crystals. Therefore, the resulting image is

rectangular. The field of view in linear-array instruments is mostly from 5-10 centimeters wide and 20 centimeters deep resulting in a scanning area of 100-200 square centimeters (61). The transducer is moved slowly from one position to the other in an area just in front of the udder. This area should ideally be shaved for effective scanning. Most operators scan only the naturally bare area of skin next to the udder. Scanning only this area could lose the opportunity of imaging some areas of interest, which can't be scanned by focusing on this area. Linear-array scanners are commonly used in domestic animals because they usually cost less than sector scanners. However, fetal number counting is more challenging than with sector probes. To obtain satisfactory results in determining fetal numbers, the ewes should be placed on their backs and the abdomen area in front of the udder should be shaved.

The sector scanners have a smaller number of crystals, which emit sound waves in an arc that looks like a "slice of a pie" on the CRT screen. This type of scanner gets its name because the image appears on CRT as a sector-shaped. The common angle of sector scanners is 90 degrees, but wider or narrower angles are available for particular purposes. Most sector scanners operate a scanning area of over 465 square cm. There is a new sector scanner that was designed in Scotland ; BCF technology(80). This instrument allows a wider area (up to 927 square cm, up to 170^{o}) of interest to be viewed on the CRT screen. This method was designed specifically for the determination of pregnancy and fetal numbers in sheep. Using this instrument allows the operators to obtain much faster and better results when diagnosing multiple pregnancy because the area, which will be viewed, is very wide. Sector scanners are used widely in the human

medical field. The image obtained by this instrument is much better than that obtained by linear-array scanners.

Real-time ultrasonic scanners are also categorized according to their particular usage. They can be used either trans-rectally or trans-abdominally. Trans-rectal scanners are used widely to diagnose pregnancy in large farm animals such as horses, and cattle. These scanners are used for obtaining an early pregnancy diagnosis. In addition, these internal probes can be used in small ruminants including, sheep, llamas and goats. The principle and techniques of using trans-rectal ultrasonography are reviewed by Deas (72). The clean , lubricated rectal probe is inserted to the lubricated rectum and slowly rotated from side to side. Then, the operator tries first to search for the distended urinary bladder which appears as a visible sized dark area .The probe is then advanced forward to view the uterine horns. The uterus of an open ewe will be uniformly gray. On the other hand, the early feature, which can be seen in the uterus of pregnant ewes, is the embryonic fluid, which is displayed as a non-echogenic area inside the uterus. The earliest indication of pregnancy in ewes utilizing embryonic fluid in the uterine horns was observed by day 15 post breeding (81).

Trans-abdominal scanning is used for diagnosing pregnancy in sheep and goats. Fowler and Wilkins (1982) were the first people who used this technique (78). Examination with both linear and sector probes can be performed in standing ewes from the right side using contact medium(water, vegetable oil, contact gel). Using the gel ensures acoustic coupling with the skin and transmission of the ultrasonic energy into the abdomen. The diagnosis of pregnancy by using this method can be done very quickly on the basis of the imaging of embryonic fluids , fetus and placentomes. The ideal time to

perform trans-abdominal ultrasonography is at 40-70 days of gestation, however, it can also be done as early as after 30 days. Over 95% diagnostic accuracy can be obtained after 40 days of gestation . Multiple pregnancy can be detected after day 51 of gestation by this instrument (82).

Real-time ultrasonic scanning can be used to diagnose pregnancy and determine fetal numbers in sheep and other species of farm livestock. Pregnancy can be diagnosed very early by imaging fluid in the uterine lumen, visual determination of the fetus (s), or by finding evidence of placentomes (83, 84, 85). Pregnancy can be detected in horses and cows as early as 9 and 10 days of gestation respectively with trans-rectal real-time ultrasonographic machine (86, 87, 88, 89). The real-time ultrasound is also used to diagnose pregnancy in the sow and gives accurate results when used after day 22 of gestation (76). In goats, pregnancy can be detected as early as in ewes (90), and the accuracy of diagnosing single, twins, and multiple fetuses at 35 days of gestation was 100%,89%, and 82% . The use of real-time ultrasound for pregnancy diagnosis in sheep was reviewed by many authors (91, 92, 19). Many trials have been done on using real-time ultrasonography to detect early pregnancy and fetal numbers in sheep. White et al. (9), conducted a study to diagnose pregnancy in 1,120 ewes between 50 and 100 days of gestation. In this study, the accuracy of detecting pregnant vs. non-pregnant ewes was 99% and they obtained 97% diagnostic accuracy in diagnosing the exact number of fetuses. Buckrell et al. used a linear - real -time ultrasound system with 5 MHz rectal transducer to diagnose pregnancy in 64 ewes (83). The accuracy of distinguishing pregnant vs. non-pregnant ewes , after 30 days of breeding, was 91% and it took less than one minute for each examination. In an other study, 95% overall accuracy was achieved

for the diagnosis of barren ewes and ewes carrying either single or multiple lambs (93). In 1988, twenty four Suffolk and two Dorset ewes were subjected to intra-rectal and trans-abdominal scanning (5). Intra-rectal scanning was perform every other day from day one until day 30 post breeding then twice weekly until day 50 of gestation while trans-abdominal scanning was used two times a week from day 25 to 55, then continued weekly until parturition. The earliest time that pregnancy was detected by intra-rectal and trans-abdominal probes was at days 20 and 25, respectively. The earliest observation of multiple pregnancy was at day 31 by the trans-rectal scanning. In this study, the diagnostic accuracy of diagnosing ewes carrying one fetus , or two fetuses was 100% and 97.3%, respectively when the trans-abdominal procedure was done between 51 to 75 days of gestation. Higher frequency transducers were applied by Schrick and Inskeep (94) when they examined 36 cross breed ewes by using a 7.5 MHz transducer (human prostate , linear -array). They used this scanner trans-rectally and the ultrasonography was performed daily from day zero (the day of estrus) and continued to day 25. Extra embryonic fluid and membranes were observed in the uterine horn by day 15 post breeding. The heart beat was first detected in day 18 or 19 post breeding. In Turkey, (95) a study was conducted by Muhammet et al. to investigate whether ewes selected for slaughter could be diagnosed by using real-time ultrasonography technique. The goal of this study was to separate pregnant from open ewes and as a result the economic loss by slaughtering those pregnant ewes can be decreased . They used a 5 MHz sector transducer and obtained 97.84% diagnostic accuracy.

The determination of gestational age by real-time ultrasound technique :

In large animals such as cows and horses, rectal palpation can be used to estimate the stage of pregnancy. However, this method can't be used in small ruminants for the same purposes because the pelvic cavities in these animals are small. The determination of gestational age in small ruminants is based generally on knowledge of the breeding date. An accurate way of determining the gestational age and the development of the fetuses in these animals was by slaughtering the pregnant females with known breeding dates and measure the size of their fetus(es) throughout the pregnancy period (96, 97). Currently, using real-time technology provides the chance of following development of the fetuses and the determination of gestational age in live pregnant females (2, 98) (99) . Human fetal age has been estimated using ultrasound measurement of fetal heart width in the last several years (100). After that, the use of this technique for estimation of pregnancy continued in variety of animal species (101, 102, 103, 104,) . In sheep, there have been many trials to estimate gestational age over the last few years. The changes in the location of the uterus, the presence of embryonic fluid, the presence of fetus(s) and placentomes and their development can be taken as a useful guide when the stage of pregnancy needs to be estimated. Kelly and Newnham (1989) made an estimation of gestational age in Merino ewes by ultrasound (105). They measured the length and the width of the fetal head at two week intervals from about day 45 of pregnancy in Merino ewes carrying single fetuses and at one week intervals from about day 36 to 86 of pregnancy in Merino ewes carrying twin fetuses . They concluded that the measurement of fetal head length by ultrasound instrument can provide a useful estimation of gestational age in Merino ewes. They found that the internal measurement of the head

length between days 40 and 80 of pregnancy offers the most accurate result in prediction fetal age . Aiumiamai et al. (1992) measured the biparietal diameter of the skull, the heart rate and the diameter of the body trunk of the fetuses of seven ewes (2). They found that there was a strong correlation between the diameter of the skull and the body trunk and the gestational age . Johns (1993) tried to estimate the week of conception in 381 Merino ewes (98). The ewes were scanned after 92 days from the introduction of rams. The determination of the stage of pregnancy was based on the measurement of the heads and the bodies of the fetuses. The result of this study showed that the operator could obtain 90% diagnostic accuracy of the week of conception in those ewes.

Pregnancy Examination of Najdi Sheep

Material and Methods:

Study Design:

B-mode real-time ultrasonography technique was used to determine the pregnancy status at three different times of gestation. Najdi ewes in groups 2 and 3 were used. The ewes were diagnosed at 20, 40, and 60 days post breeding.

Ultrasonic machine:

The real-time ultrasonic scanning instrument used was Concept / MCV (Figure 7 and 8). It is a portable scanner capable of imaging with linear, convex and microconvex probes. In this study a 5MHz trans-abdominal linear transducer was used.

Figure 7: The ultrasonic machine

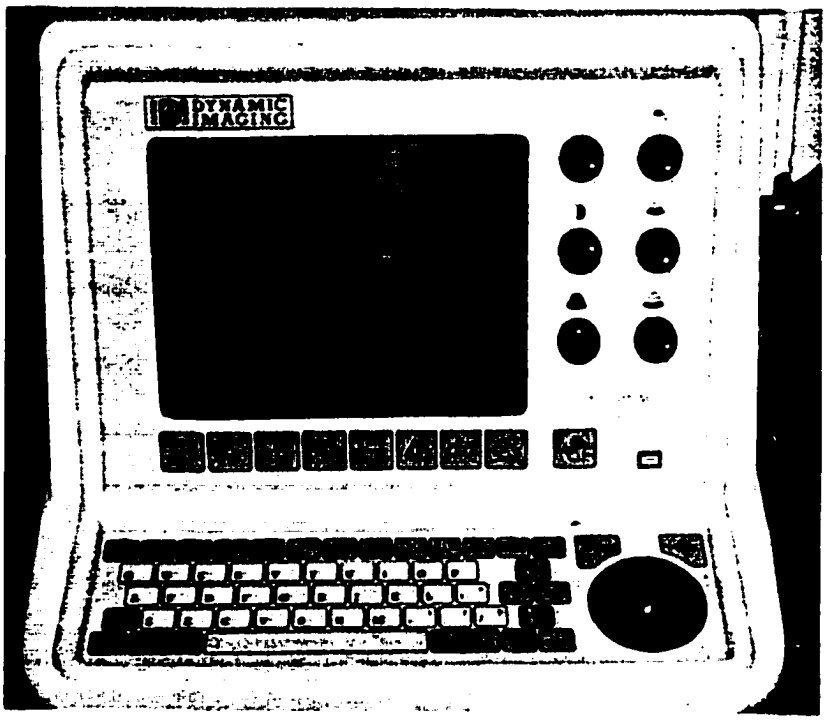
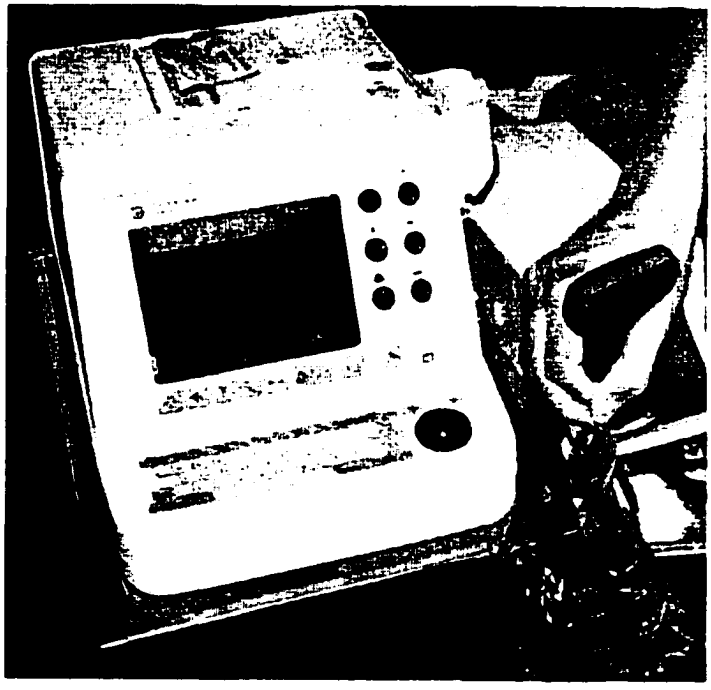


Figure 8: The ultrasonic machine and the 5 MHz transducer



Procedure:

The ewes in groups 2 and 3 were examined at three different times, 20,40 and 60 days post breeding. Each time, the ultrasonic examination was conducted with the ewes in a standing position. The ewes were restrained in a chute where the ambient light was bright. Therefore, a large sheet of cardboard was put over the ultrasonic machine to decrease the effect of sunlight. The ewes were held by a worker. A commercial lubricant was applied, just to the right of the area in front of the udder deviod of wool, to insure satisfactory acoustic coupling. The ewes were scanned by sweeping the right wool-less area with the trans-abdominal transducer.

At the first examination 20 days post breeding, the examiner tried first to locate the uterus and observe any change in its size. The observation of embryonic fluids, which appears as a dark area inside the uterus, was taken as an indicator of pregnancy. In addition, visualizing the embryonic vesicle fluids or the amnion were taken as a sign of pregnancy. A small uterus with no embryonic fluids was taken as an indicator of an open ewe.

At the second examination, 40 days post breeding, the examiner tried to observe the placentomes or fetus itself. At this stage, heartbeat and fetal movement were taken as evidence of having viable fetuses. At the last third examination, 60days post breeding, the operator tried to locate the fetus and its parts. The present of the placentomes also was examined. Ewes were examined only to determine if they were pregnant or not. No attempt of determining fetal number was done in this study.

The average ambient temperature at the first, second and third examination were recorded. The statistical analysis was done by using Minitab software. Proportion test

was used for determining the accuracy of pregnancy diagnosis at each examination. The accuracy of pregnancy diagnosis was assessed on the basis of lambing results.

Results:

Of the 193 Najdi ewes. 102 gave birth. Table 1,2 and 3 show the result of the ultrasonic diagnosis at day 20,40 and 60 of gestation. The total time spent in examining the ewes in first, second, and third examinations were 3.5, 3, and 2.25 hours, respectively.

Table 1: Results of ultrasonography examination at 20 days:

		ACTUAL LAMBING RESULT		
		TOTAL	PREGNANT	OPEN
US* RESULTS	PREGNANT	67	52	15
	OPEN	126	50	76
	TOTAL	193	102	91

US* = Ultrasonographic

Table 2: Results of ultrasonography examination at 40 days:

		ACTUAL LAMBING RESULT		
		TOTAL	PREGNANT	OPEN
US RESULTS	PREGNANT	99	91	8
	OPEN	94	11	83
	Total	193	102	91

Table 3: Results of ultrasonography examination at 60 days:

		ACTUAL LAMBING RESULT		
		Total	PREGNANT	OPEN
US RESULTS	PREGNANT	102	102	0
	OPEN	91	0	91
	Total	193	102	91

At day 20, enlargement of the uterus plus the presence of embryonic fluids were observed in 67 ewes. The embryonic fluids appeared as a dark area inside the uterus. These embryonic fluids were not seen in the rest of ewes, which were called as non-pregnant. The embryo and the placentomes were not observed at this stage. At 40 days of gestation, the examinaer was able to observe the fetus, which was as a white object floating inside the embryonic fluids. In addition, placentomes were observed in several ewes. At 60 days of gestation, the uterus was larger and several parts of the fetuses were easily observed. Fetal head, body, ribs, limbs, stomach, and heartbeats were observed at this examination.

At the time of ultrasonic examination, the ewes had already been fed. The examinations were done in late morning where the ambient light was bright. The average ambient temperature in the first, second, and third examinations were approximately 35, 39 and 41^{o}C respectively. The accuracy of pregnancy diagnosis based on lambing results were 66.32, 90.16, and 100% when the real time ultrasonic examination was applied at 20, 40, and 60 days post breeding.

Discussion:

The ability to predict pregnant from open ewes at early stage of gestation with high degree of accuracy can provide many benefits to the sheep producers. Open ewes can be early rebred or culled. As a result, the producers will have an idea about the number of lambings they will have; therefore, they will have much time to prepare themselves for lambing. In addition, if fetal numbers are determined, they can manage ewes differently depending on the number of lambs they carried. This will assure providing the right nutrient requirements for each ewe. The correct feeding of ewes at the last month of gestation and during lactation affect the post partum interval and milk production.

In this study, the accuracy of pregnancy diagnosis by using 5MHz trans-abdominal linear transducer increased when the machine was used 40 to 60 days post breeding. By day 20, 67 ewes had fluid in their uterus and so were classified as pregnant. Fifteen of these 67 ewes did not subsequently lamb. Those 15 ewes might have had an early embryonic loss due to the high ambient temperature during the first 20 days of pregnancy, more than 32c. Increasing the ambient temperature from 23-32.2 C cause very high embryonic wastage when this increasing took place at the early cleavage stages (106). The most sensitive period of embryonic development to high environment temperature is between days 1-16 of fertilization.

The accuracy of ultrasonic diagnosis of pregnancy would be higher if the following were applied:

1)- the ewes were diagnosed before feeding.

2)- the diagnosis was done in a dark place or under a shed.

3)- a trans-rectal transducer with higher ultrasonic frequency was used.

Finally, we conclude that real-time ultrasonography can be used for pregnancy diagnosis in Najdi ewes. In this study, pregnancy was determined with relatively low accuracy at 20 days of gestation. However, higher diagnostic accuracy can be obtained when the examination takes place 40 (90%) or 60(100%) post breeding. At these advanced stages the presence of the fetus or any of its parts and the placentomes are easy to be observed and give high assessment of pregnancy.

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CHAPTER 4
ECONOMIC ASPECTS OF THIS STUDY

Introduction:

Serious agricultural development in the Kingdom of Saudi Arabia began in the 1970s when the government launched extensive programs promoting modern farming technology. These programs encourage agricultural research, enterprises, and training-institutions. Intensive dairy, meat, poultry, and egg farming were all introduced early in those programs. The Kingdom has accomplished self-sufficiency for many animal products. In addition, the Kingdom has started to export some products such as dairy products, eggs, fish, and poultry. However, lamb production is still below that needed for the local demand. Saudi Arabia imports more than 3 million live or slaughtered sheep from other countries.

There is a belief in Saudi Arabia that sheep enterprises are not profitable. Several sheep projects have been closed because the income from raising sheep doesn't cover costs. The prices of feed are high and lamb marketing is changeable. In addition, the production ability of local breeds is low and management of these sheep is not optimal. In addition, the local sheep show especially low reproductive performance at the end of spring and during summer months.

In this study two reproductive management techniques were used during the low reproductive periods. The economic aspect of using these two methods in regard to ram and ewe costs are theoretically discussed below (1, 2) :

1)- **Cost of ram per ewe :**

Ram initial cost = $ 200

Annual depreciation (5 years) = $ 40

Feed and pasture = $ 88

Death loss (9%) = $ 18

Vet. Cost = $ 12

Labor = $ 15

Overhead = $ 7

Annual cost = $ 180

Annual cost of ram/ewe

ram : ewe

Group 1 : 8 : 100 = 8 X 180 = $ 1440

Group 2 : 10 : 100 = 10 X 180 = $ 1800

Group 3 : 4 : 100 = 4 X 180 = $ 720

Ram cost / ewe in group 1 = 1440/100 = $ 14.4

group 2 = 1800/100 = $ 18

group 3 = 720/100 = $ 7.2

Cost for treating ewes in group 2 and 3 :

Cost for CIDR/ewe = $ 3.20

Cost of CIDR insertion and removal / ewe = $ 0.50

Cost for PMSG injection / ewe = $ 1.0

Cost for AI (labor + extender + straw) / ewe = $ 1.0 (excludes technican costs)

Net cost / ewe

Group 1 = $ 14.4

Group 2 = 18 + 320 + 0.5 + 1.0 = $ 22.7

Group 3 = 7.2 + 3.20 + 0.5 + 1.0 + 1.0 = $ 12.9

2- **Cost of rams / lamb:**

Number of lambs born from group 1 = 35

Number of lambs born from group 2 = 76

Number of lambs born from group 3 = 70

Cost of ram / lamb in group 1 = 14 X 100/35 = $ 41.14

group 2 = 22.7 X 100/67 = $ 33.88

group 3 = 12.9 X 100/70 = 18.45

This calculation shows that the cost of a ram for each lamb born were lower in group 2 and 3. It was the lowest in group 3 when few rams were used. In addition, the cost of managing ewes in group 2 and 3 would be lower because of the fact that these synchronized ewes had a shorter lambing period. Finally, the cost of raising lambs of group 2 and 3 that relatively had same age would be lower.

Cost of Najdi ewes:

Good management and high production of ewes are essential for getting high economic return for raising them. Management of non-reproducing ewes decreases economic return. High production of ewes is essential for getting high economic return for raising them. Non-producing ewes can't even cover the cost of their maintenance and will affect the net profit of a sheep project. For this reason, the economic reward of keeping ewes in their groups will be explained :

Current records at NADEC sheep Unit indicate:

Multiple birth % = 15

Average Marketing lamb weight (lb) = 88

Price of live lamb / lb = $ 1.57

Annual cost / ewe = $ 80

Average cost of lamb from lambing until marketing = $ 50

Price / lamb = 88 lb X 1.57 = $ 138.16

Net price = 138.16 - 50 = $ 88.16

If all ewes lamb with 15% multiple birth

Therefore, number of lambs / 100 ewes = 115

Net profit if all ewes are lambed = 115 X $ 88.16 = $ 10,138.4

Net profit from each ewe = $ 10138.4 / 100 = $ 101.38

Average ewe cost 80

Lost opportunity 101.38 +

Lost profit / open ewe $ 181.38

Ewes did not lamb in group 1 = 69

group 2 = 47

group 3 = 51

Total lost from group 1 = 69 X 181.38 = $ 12, 515.22

group 2 = 47 X 181.38 = $ 8, 524.86

group 3 = 51 X 181.38 = $ 9250.38

It is obvious that the total loss from ewes in group 1 is very high when compared with the other groups.

Economical return from early pregnancy diagnosis:

The benefits of diagnosing pregnancy in sheep has been discussed in chapter 3. One of these is the economic advantages, which can be achieved especially if the diagnosis is done early with higher accuracy rate. In this study, higher trans-abdominal ultrasonic diagnosis (100%) of pregnancy of Najdi ewes was obtained after 60 days of breeding . At this farm, the ultrasonic examinations take place at 90 days of gestation. Therefore, culling non-pregnant ewes is done 90 days post breeding. This means that the cost of maintaining open ewes can be decreased if they diagnosed after 60 days from breeding.

Annual cost of ewe/ year ($) = 80

Cost of ewe/ month ($) = 80/12 = 6.66

In this study, for groups 2 and 3 the number of ewes that were correctly diagnosed as non-pregnant at 60 days = 91

Amount of dollars that can be saved when these ewes are culled a month earlier($) =

91 X6.66 = 606.06

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