An-Najah National University
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Different Estrous Induction Protocols During the Non-Breeding Season in Assaf Ewes

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Dedication

To my mothers, father, sisters and brothers.

To my wife and my family

I dedicate this project

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الإقـــرار

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أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

تحفيز الشبق بطرق مختلفة لدى اغنام العساف خارج موسم التناسل

أقر بأن ما اشتملت عليه هذه الرسـالة إنما هو نتاج جهدي الخاص، باسـتثناء ما تمت الإشارة إلية حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: .....**:اسم الطالب**

Signature: .....**:التوقيع**

Date: .....**:التاريخ**

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List of Abbreviations

GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
PGF2α	Prostaglandin F2α
EIA	Enzyme immunoassay
RIA	Radioimmunoassay
E2	Estrogen
FGA	Fluorogestone acetate
eCG	Equine chorionic gonadotropin
MAP	Medroxyprogesterone acetate
PMSG	Pregnant mare serum gonadotropin
P4	Progesterone
FSH	Follicle stimulating hormone
hCG	Human chorionic gonadotropin
I.U.	International Unit
CL	Corpus Luteum
AVCP	Advanced Veterinary Manufacturing Company Palestine
SPSS	Statistical Package for the Social Sciences
DAS	Digital and analog system

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Different Estrous Induction Protocols During the Non-Breeding Season in Assaf Ewes

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Abstract

This experiment was conducted to investigate the effect of pregnant mare serum gonadotrophin (PMSG) levels (300 or 600 I.U.) and the progestagen method (one sponge for 14 d or two sponge each for 7 d) on estrus response, onset and duration of estrus,lambing rate, litter size and serum progestagen concentrations during estrus period and early pregnancy. Atotal of 20 assaf ewes were used in the experiment which was conducted during April, a month that is considered as non-breading period in Palestine. Ewes were inserted with intravaginal sponges that containing 60 mg Medroxyprogesterone acetate (MAP). Seven days later, these sponge were removed and 10 new sponges were inseted to 10 of the experimental ewes. Following withdrawal of sponges, at day 14, 5ewes from each tretmant groups were injected intramauscularly with PMSG at level of 300 and 600 IU.

This investigation showed that level of PMSG and progesterone application methods had no significant effects on the tested parameters, also, there are significant different in progesterone concentration between ewes gave brith to single and twines. This finding indicated that low level of PMSG can be

applied for estrus synchronization where lots of savings can be achieved for the benefit of local sheep farmers. Although, some slight differences were obtained but there was no significant differences between the two different progesterone application methods nor the two different PMSG doses. So one sponge followed by 300I.U. PMSG can be used to synchronize estrus successfully and with lower cost than using two sponges or high PMSG doses.

Chapter one

Introduction

Sheep production is one of the most important agricultural sectors in Palestine. The sheep population was estimated to be about 744 thousand heads (Palestinian Central Bureau of Statistics, PCB, 2008). The sheep population is composed of the Awassi breed which comes in the first place followed by the Assaf then the improved Awassi breeds.

Assaf breed, the breed under focus, is the hybrid produced by the crossing of Awassi with the East Friesian breed. It is a high milk producer with annual production of 300 to 400 liters (Pollott and Gootwine, 2004). Assaf breed is most suitable for intensive farming and it is an early maturing breed.

Currently, Assaf ewes are usually maintained under an intensive management system, where ewes are milked after lambing, and lambs are transferred at birth to artificial rearing units. While in palestine this management method is not widely applied (Pollott and Gootwine, 2004**)**.

Over the past few decades the main achievements in improving the reproductive performance of sheep have been in the control of reproductive diseases. However, great wealth of information has been accumulated on the physiological control and interaction of reproductive system, in addition, the science of application of exogenous drugs is entering a new phase, there is concentration on the development of slow releases delivery systems, particularly for hormones, which may more closely mimic the effects of natural hormone production and therefore hopefully be more effective.

Reproductive is an important in the field of animal production, in order to maintain the type and the continuation of production. It is documented that not less than 60% of local farm profits comes from selling lambs, this commodity is influenced by the lambing rate, lambing interval and the reproductive efficiency in general. The majority of sheep breeds differ in reproductive behavior depending on season changes, latitude/longitude, the length of the photoperiod and other factors.

In Palestine is located along the line 14-34 and 40 - 35 east and between latitudes 30-29 and 15-33 in the north. The peak of ewes breeding activity occurs in the period from September to November and lambing activity in months of February to April.

Breeding and lambing patterns are reflected in seasonal availability of lamb and fluctuations in price. Incentives to breed ewes more than once per year include reduced costs per offspring reared, increased net return. Because sheep are seasonally polyestrous, an attempt to mate at a frequency greater than once a year will require one breeding season during or near anestrous, without intervening treatments during anestrous, little ovarian and estrous activity occurs, and pregnancy and conception rates are low, especially if out-of-season breeding occurs during the early postpartum period. The major problems that faces the farmer sheep in Palestine is reproduction management (estrus synchronization), this is necessitates looking for reproductive techniques, which could help the farmer, and decrease the cost of production.

Different methods of estrus synchronization were investigated worldwide, however, this was never performed in Palestine.

In order to achieve this goal, Progesterone or a progesterone analogue is usually used to synchronize estrous in sheep during the breeding and non-breeding season.

Intravaginal used sponges have some side effects such as vaginal contamination and infections when used for long periods, also the hormone contained in the sponge may be diminished with time.

The objectives of this study are to investigate the effect of refreshment intravaginal sponges and to investigate the effects of PMSG levels on ewes synchronized, and to the effect of PMSG with inravaginal sponges on the reproductive parameter to estrus response, onset and duration of estrus, lambing rate, litter size, serum progesterone concentration for estrus detection and pregnancy diagnoses and determination of animal reproductive state.

Chapter Two
Literature Review

I. Ovine reproduction

I.1 Physiology of Reproduction

I.1.1. Seasonality and Ovarian Activity

One of the most special characteristics of ovine reproduction is seasonality. Most breeds of sheep will breed during a restricted time period during the year, Therefore, their reproduction follows a seasonal pattern by alternating periods of anoestrus and periods sexual activity (Notter, 2002) during the periods of sexual activity they exhibit multiple estrus cycles until the end of season or to become pregnant, so they are intermittently polyestrus or seasonally polyestrus animals (Bearden and Fuquay, 1984).

In temperate regions the day length has a dominant controlling influence on initiation and termination of breeding season, their breeding season is initiated as the ration of daylight to darkness decreases and end when increasing day lengths reach a ratio of nearly equal daylight and darkness, short days inducing anoestrus, so some times they are classed as (short-day breeders) in contrast to (long-day breeders) such as the mare(Yeates, 1949; Legan& Karsch, 1980, Robinson and Karsch,1987).

In seasonal breeders, it is theorized that the retina of the eye is the sensor for the light signals, with impulses going by way to the optic nerve and other nerval pathways to the pinal gland (Notter, 2002).

Probably, sheep detect day light changes by means of a bult-in biologic clock in the hypothalamus, and this information is transmitted to the hypothalamus gonadal axis via the pineal gland (Bowen, 2003). Melatonin, a pineal hormone, mediates the response to changes in the photoperiod in

sheep (Bowen, 2003), melatonin levels are high during dark period and low during light period, probably these differences in the pattern of melatonin secretion act as a signal indicating day length to the nevroendocrine axis (Jordan, 2005), melatonin which is produced and secreted by the pineal gland exclusively at night, is believed to determine the effects of photoperiod on the cyclic activity of the ewe, along duration of melatonin secretion signals a short-day, where as a short duration of melatonin secretion signals a long day (Younes, 2008).

While photoperiod is the main determined of the seasonality other factors can influence reproductive pattern such as genetic (some breeds are more resistant to light variation), management practices (male effect) and social causes (Wingfield et al, 1983, Deviche and Small, 2001).

In the tropical zones, where there is less variation in day length, sheep tend to breed throughout the year, therefore, when temperate breeds are introduced into the tropics, they gradually lose their seasonality and follow the breeding patterns characteristic of the new environment, also movement of sheep between northern and southern hemisphere is followed by adaptation to the appropriate breeding season (Rodrigues et al, 2007).

Genotype in fluencies the seasonality and sexual activity in the ewe. With some ewes showing estrus year- around, the Dorset, merino, and rambouillet sheep breeds, which originated near the equatorial, have longer sexual season than British breeds.

Seasonality not only effects mature animals, it can also influence the age of puberty. although genetics plays major role in the age which animals reach

puberty, the date of birth and therefore photoperiod can either have a permissive effect, allowing the animal to come into estrus at an early age, or delay puberty for several month, for example, ewes born in late summer will be older at puberty than those born in early spring or late winter (Foster, 1981).

Whilst rams are able to mate at any time of the year, they are effected by photoperiod, showing highest breeding activity and fertility in the fall . Reduced sperm production, more abnormal spermatozoa, and lower fertility are characteristic of rams as photoperiod lengthens as in spring and early summer (Fitzgerald and Stellflug, 1991).

Sheep breeds that live in the Mediterranean region generally showing cyclic activity occurring between late June and early April, with a peak breeding activity usually falls between September and January (Bearden and Fuquay,1984). So that the young are born during the most favorable time of the year.

Estrus activity stops with pregnancy and is not resumed for some time after lambing, due to the called(post –partum anestrus) .The post-partum anestrus is mainly due to the antigonadotrophic effects exerted by the suckling lamb so it normally disappears shortly after weaning (Bearden and Fuquay, 1984).

I.1.2. The Estrus Cycle

Non pregnant ewes will shown estrus cycles or sexual activities at regular intervals during their breeding season.

The length of estrus cycle is 16-17 days with a range of 14-19 days (Hafez, 1966, Patterson, 1968), however, in the transition period between anestrus and breeding season, short cycles of less than 12 days, are particularly common, generally the first ovulations of the breeding season are not accompanied by estrus behavior and are know as silent estrus.

As in other species the estrus cycle can be divided into phases. the follicular phase 3-4 days, and the luteal phase that lasts about 14-15 days, which characterized by the maturation of the corpus luteum and high levels of progesterone that reach a maximum peak about 6 days after ovulation (Jordan, 2005).

On the day before estrus one or more follicles grow rapidly and the concentration of 17-B estradiol in the blood increase from about 10 to 20 pg\ml (Błaszczyk et al, 2004), the estrogen causes behavioral estrus, and increased estrogen levels are stimulation of cervix to secret small amount of mucus, increase vascularization in the vulva and some changes in the epithelial tissue of the vagina (Hafez, 1980a, Hafez, 1980b, Anderson, 1991, Theodosiadou et al, 2004).

The synergic action of estrus of estrogen with Gonadotropin Releasing Hormone (GnRH), stimulate release of luteinizing Hormone (LH) from anterior pituitary gland. the concentration of LH in the blood rises to a peak

after the beginning of estrus , and then both LH and estradiol concentration fall rapidly (Younes, 2008). Lutelizing Hormone stimulates ovulation, which occur about 14 hours after the LH peak, or 24 hours after the beginning of estrus (Pierson et al, 2001, Alvarez et al, 2007), but throughout the other period of the cycle, the LH concentration remains low (2 to 3 ng\ml).

The duration of estrus varies with age, breed, presence of the male and season ranging between 18 and 72 hours with an average around 36 hours. Wool breeds have longer estrus period than meat breeds. Estrus duration is shorter at the beginning and end of the breeding season, in the presence of the male, and in the first breeding season of the young females (Hashemi et el, 2006).

Ewes are spontaneously ovulates, they normally ovulates near the end of estrus about 24 to 27 hours after onset of estrus (Bearden and Fuquay, 1984) . Compared with other ruminant females, estrus in the ewe is less apparent .If a ram is present ewes in estrus will seek him out, may display tail- wagging and nuzzle his scortum. If the ram tries to mount them they will stand to be mounted, but if no ram or an inexperienced one is present estrus can remain undetected (Bearden and Fuquay, 1984).

Ovulation rate(number of eggs released at ovulation) is influenced by a number of factors including breed, age, reproductive status (dry or lactation) , season of the year, nutritional status and body condition of the ewe.

In many breeds of sheep two or more ova are released during each estrus. The ovulation rate is 1.2 for merinos (Driancourt et al, 1990), the twining rate for Assaf 1.57 (Pollott and Gootwine, 2004). The ovulation rate increases with age and reach a maximum at 3 to 6 years, declines gradually (Schoenian and. Burfening, 1990).

Among the environmental factors influencing ovulation rate, season and levels of nutrition are important. Generally, ovulation rates are higher early in the breeding season than later (Lamberson and Thomas, 1982). Flushing or increasing the levels of nutrition before mating, is commonly practiced in sheep to increase ovulation rate (Johonson et al,1990, AbuEl-ella, 2006), but factors such as body size, weight, condition, and genotype may also contribute to the increase in ovulation rate.

If no embryo is present in the uterus on the twelfth day, the corpus luteum then regresses, and the estrus recur. On the twelfth day in the non pregnant and pregnant ewe, prostaglandin F2α (PGF2α) increase in concentration. in the non pregnant sheep PGF2α reaches a peak on the fifteenth day of a concentration in the uterine vein of about 10ng\ml. PGF2α is known as the luteolysin in the sheep and reaches the corpus luteum by means of the close apposition of the uterine vein and ovarian artery. the CL of the pregnant ewe requires more PGF2 to cause luteolysis, this relative resistance might be produced by prostaglandin E2 from the uterus.

The corpus luteum starts to regression on the fifteenth day. By day 16 the concentration of progesterone is basal (<0.2 ng\ml). The fall in progesterone concentration stimulates estradiol secretion by the growing

follicles, and the absence of progesterone permits stimulation of gonadotropin release by estradiol.

If the conception is occur, the gestation period of sheep lasts about 5 months, 145-152 day on average. It length mainly with breed, parity and litter size (Domingues et al, 1991, Timurkan and Yildiz, 2005).

The first third of pregnancy is luteodependent, but after about the 50 the day of pregnancy progesterone is mainly produced by the placenta In consequence ovariectomy or administration of luteolytic doses of prostaglandin F2α do not terminate pregnancy during the last two thirds of gestation.

I.2 Reproduction Management

I.2.1 Introduction

Management plays an important role in the reproductive efficiency obtained females. Unfortunately reproductive efficiency approaching 100% is not possible even with the very best management, however, poor management can result in decrease in reproductive efficiency. Reproductive management can increase the lambs weaned per ewe per year as well as improve lamb marketing by increasing production of lamb in the breeding seasons and non breeding seasons.

I.2.2 Pregnant Diagnosis

The pregnancy diagnosis is considerable economic value to sheep industry (Gearhart et al, 1988, Karen, 2003), however, separation of the sheep flocks into pregnant and non-pregnant will lower the abortion rate or stillbirth and production of weak lambs (Wani et al., 1998, Karen, 2003).

However, there are differences methods for pregnancy diagnosis such as management method (non-return estrus) radiography (Karen, 2003), progesterone assay (Skemesh et al, 1973), and ultrasonography (Medan et al, 2004).

The accuracy of pregnancy diagnosis and determinant of fetal number by using the radiography method are 90% to 100 % (Ford et al, 1963, West, 1986, Karen, 2003).

Progesterone assay, concentration progesterone in blood was determined in ewes at day 18 by enzyme immunoassay (EIA) and radioimmunoassay (RIA) (Karen, 2003), the accuracy in diagnosis pregnant are 100% at 17 to 19 days (Wani, 2010), and serum progesterone values higher than 1 ng/ml. (Wani, 1989; Shreif, 1997, , Al-Merestani et al, 1999, Zarkawiet et al, 1999, Boscas et al, 2003, Wani, 2010).

Three types of ultasonography systems were used for pregnancy diagnosis in the sheep (Karen, 2003). The first A-model ultrasound, in this system the transducer containing one crystal emits, ultrasound waves penetrate the tissue under the skin(Karen,2003). however, Watt et al,(1984) reported

97% accuracy after 50 days of gestation, the system are quick, convenient and simple technique but it can not determinate the fetal number(Watt et al,1984).

The Second, Doppler ultrasound system that detect the fetal heartbeats and flow of blood in uterine and fetal vessels (Karen, 2003). The accuracy for diagnosis pregnancy at 60 days after breeding are 100% (Ishwar, 1994).

Third, B-model ultasonography system that are high accurate , rapid determinant the fetal number and estimate the age of the fetus, the accuracy of diagnosis pregnant at 21 days after mating are 80% (Medan et al, 2004).

I.2.3 Estrus Detection

Ewes do not demonstrate any signs of estrus when separated from the ram, When the ewe cannot hear, smell, or see the ram, this causes diminished estrus behavior, characteristic behavior for the ewe is rapid tail movement or raised tail in the presence of the ram, there are secondary characters such as nervousness, walking the fence, and a decrease in milk production and appetite, there are Physical characteristics include a reddened, swollen vulva, but this is often difficult to detect because of wool and small size of the vulva (Yager et al).

There are different methods used for detection of estrus such as state of vaginal cells and cervical mucus (Zourgui and Elze, 1976).And electric resistance of the vaginal mucus (Abdel Rahman and El nazier, 1987).

I.3 Control of Estrus Cycle

I.3.1 Introduction of Rams

This method used to achieve breeding activity during the non-breeding season, which the anestrus ewes are previously isolated with rams before the start of normal breeding season, introduction of rams to ewes inducing ovulation, this method is referred to as the ram effect or male effect (Jordan,2005). The ram effect allows induction breeding during anestrus and produce some of synchrony of cycle among the ewes in flock (Chanvallon et al, 2008). However, this common practice to isolate the ewes from rams (including sight, sound, and smell) for a period of time before introduction to get reproductive response (Jordan, 2005).

In anestrus ewes decrease secretion of GnRH and LH compared in ewe during breeding season. In additional, the first effect of introduction of ram to ewes increase secretion LH and causing onset follicular phase and increase secretion estrogen E2 (Rosa and Bryant, 2002). After of introduction of ram LH surge within as little as 6 to 8 hours. However, the ewes are continually contact with ram take more time to being mating than isolated ewes stimulated to breed by the sudden introduction of rams in late anoestrus (Rosa and Bryant, 2002).

The ram effect in anestrus season is less effective to induce the cycle when is alone. Some researches used progesterone treatment with ram effect this to investigate interaction between ram effect and control breeding programs

(Romano et al, 2001), and cycle response (Vasques et al, 2006). Romano et al,(2001) found when used ram effect continually with ewes which treated 30 mg fluorogestone acetate (FGA) for 14 days and 200µg prostaglandinF2α to shorten onset of estrus (32.9±1.6h)compare intermittent presence of the ram (45.3±4.4h), and reduces the interval between sponge removal and ovulation, this agreement with Vasques et al(2006) when used 45 mg FGA for 12 days and 500 IU eCG and introduction of ram, found shorten in onset of estrus low than 44 hours from control group 48 hours.

I.3.2 Progestagens

Estrous synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency, particularly in ruminants(Kusina et al,2000, Hashemi et al, 2006), and can be improving sheep production systems by nutritional and\or hormonal treatments resulting in higher estrus responses and subsequent conception rates (kridli et al.2003). The general hormonal technique for estrous synchronization in ewes is the use of intravaginal devices impregnated with progesterone or synthetic progestagen such as fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) (Fukui et al, 1999, Karaca et al, 2009). The Intravaginal sponges impregnated with progestagen have been widly used in sheep and during the breeding and non-breeding season (Corteel, 1973, Robinson, 1979, Corteel et al, 1982, Romano, 2004).

Intravaginal sponges are usually inserted over periods of 9 to 19 d and used in conjunction with pregnant mare serum gonadotropin (PMSG), particularly for out-of-season breeding, injected at time of sponge removal or 48 h prior to sponge removal, females usually exhibited estrus within 24 to 48 h after sponge removal (Wildeus, 2000).

There are problems associated with controlled breeding such as the limitation of the time and degree of estrous response. Thus, if one can pre-determine the time from withdrawal to onset of estrus, the need for estrous detection could be reduced or even eliminated (Corteel, 1975; Britt, 1987, Romano, 2004). There are also other problems linked with the period of the sponge remaining inside the vagina, the concentration of progesteron(P4) will be higher during the first 2days of insertion sponge in vagina and then decreases gradually with time during the remaining the sponge (Husein et al., 1998; Husein and Kridi, 2002; Yavuzer, 2005, Amer and hazzaa.,2009). Therefore, the amount of P4 absorbed and P4 levels at the time of sponge removal in a 12-day treatment may not be sufficient to maintain normal patterns of follicular growth (Gordon, 1975, Amer and hazzaa, 2009). Some studies considered that the use of syensitic progesterone accelerated the mechanism of follicular growth and development (No¨el et al, 1994, Kridli and Al-Khetib, 2006). However, decreased periods of progestagen treatment to facilitate management, and possibly minimize vaginal discharge and infection, and increase fertility (Fonseca et al, 2005), and minimize the period of sponges application in the vagina from 12-14days or 6days, and reducing the period of sponge insertion may maintain higher

P4 levels upon removal of pessaries may increases fertility and may reduce the chance of vaginal contamination(Amer and hazzaa,2009).

Romano, 2004, used three external sources of progesterone to compare their efficacy on estrous response, onset of induced estrus, estrous duration and fertility, The three methods were intravaginal sponges impregnated with progestagen (FGA and MAP) and CIDRs impregnated with natural progesterone during the breeding season, he found that no significant difference in estrus response and fertility between treatments.

Simonetti et al, 2000, used intravaginal sponges containing different doses 40,50,60 mg of (MAP) for 14days,he found the time of interval from sponge removal to estrus onset in lower dose of progestagen exhibited an earlier estrus response. While other researchers such as Amer and hazzaa (2009) used 40mg of FGA sponges for 6or 12 days without gonadotropin hormone such as eCG and 40mg FGA sponges for 6 or 12days and eCG (500IU) to evaluate the effects of intravaginal sponge on the reproductive performance and fertility rate of Rahmani ewes during anestrous season, found using intra-vagina FGA in 12 days with eCG adequate to improve the reproductive performance in the ewes and possible use FGA in 6 days with eCG but fertility are lower. Other used MAP or FGA or implants containing norgestomet ,for a period of 10-16 days has been successfully for estrus synchronization in the sheep, during breeding and non-breeding season(Boscos et al, 2002).

Some research's aim to investigate the efficiency of synchronization using different progesterone treatments outside the natural breeding season.

Hashemi et al, 2006, used 20 mg progesterone acetate in oil every day for 12 days, CIDR impregnated (0.3 g P4) for 12 days and 60mg MAP for 12 days and following intramuscular injection 500 IU eCG, found when used CIDR and MAP give higher effectiveness to estrus synchronization for ewes (Hashemi et al, 2006).

Other researchers such as Greyling and Van der nest (2000) used different progestone levels in natural season they used 60and30mg MAP for 14 days and followed injection of 300IUfrom PMSG,found they that there is no attribution in synchronization efficiency related to different doses of progesterone levels (Greyling and Van der nest, 2000).

Ataman et al, 2006, used progesterone treatment consisted of a vaginal sponge containing 30 mg (FGA) for 7 or 12 days and injection of prostaglandins after sponge remove during breeding season and anestrus season, he did not find any differences in during breeding season in both 7 or 12 days to pregnant rate, estrus, lambing and litter size, during anestrus season , found the percentage of estrus, pregnant, lambing rate and litter size in 7days progesterone treatment 86.6%, 76.9%,61.5% and 1.5and in 12 days treated are 93.3%, 85.7%, 71.4% and 1.5, therefore, when use progesterone treatment for 7 days was effective to synchronize oestrus in sheep during both breeding and anestrus season(Ataman et al, 2006).

Others researches used different doses of progesterone (MAP 60mg, 30mg) and 300IU PMSG at withdrawal of sponge in two breeds from goats, found no different in the efficiency of synchronization in different breeds, but the interval of estrus after remove of sponge until induce of the estrus are

shorter when used 30mg from MAP, the reason for the delayed time to incidence the estrus of 60mg MAP may be to more residual progesteron (Greyling and van der nest, 2000).

In additional, Dogan, (2006), used MAP alone and MAP\$PGF_{2\alpha}$ and MAP\PMSG and MAP\PMSG and $PGF_{2\alpha}$ found when used MAP\PMSG the mean of onset of estrus are shorter 31.1± 6.2 compared to other treatments, the duration of estrus are shorter in MAP\$PGF_{2\alpha}$ treatment compared to MAP alone treatment, this due to found high concentration from estrogen in blood after induced luteolysis and stimulation follicular growth by FSH or PMSG. However, high levels of estrogen concentration are responsible with prolonger duration of estrus (Ahmed et al, 1998, Dogan, 2006).

I.3.3 Prostaglandins

Prostaglandin (PGF2α) used synchronize estrus by terminating the luteal phase through regression of the corpus luteum, prostaglandin-based systems used only during the breeding season. Because not all stages of the estrus cycle are similarly receptive to prostaglandin treatment (Ataman et al, 2006).

The most used methods of PGF2α are the first one is to use progesterone impregnated intravaginal sponges for 7 or 12 days following by a single prostaglandin injection, this method can be use in during season and anestrus season, another method is applied by two injection of

prostaglandin at 11 days interval, this method can be used only during breeding season. (Ataman and Aköz, 2006).

Other research's used GnRH-PGF2α compination they found that using GnRH with PGF2α was effective in the synchronization of the oestrus in ewes (Ataman and Aköz, 2006). Also, Szbülür et al, 2006, used double injection of 125 µg of Cloprostenol (a PGF2α analogue) at 10 or 14 days intervals to detection the oestrus and lambing rates in Tuj ewes during the breeding season, they found petter results when using the 14 days interval. (Sozbülür et al, 2006).

I.3.4 Melatonin

Melatonin, which called the darkness hormone, secreted from pineal gland, secretion increase when the day length becomes shorter, which effect on hypothalamus and secretion of the gonadotropin hormone, However, can be using exogenous melatonin as mimic effect to shorting day lengthy, therefore, used widely to improve performance of reproductive during anestrus (Jordan, 2005).

Numerous experiments have tested various protocols involving different administration of melatonin and duration of treatment with melatonin and have produced varied results, such as Stellflug et al, (1988) used different concentration of melatonin(2 or 10 mg) and routs of administration fed or implanted and duration of treatment (20 or 40) days in late March and April, found when using 2 or 10 mg or implant for 40 days that enhanced

reproductive performance which synchronized estrus was increased and lambing in mature ewes. Carlson, (2000) used melatonin and progesterone, he found that melatonin implant increased pregnancy rate in anestrus season compare to non-treat control, O'Callaghan et al, (1991) found that melatonin implant can affect the timing of reproductive season of the ewes by shortening the photoperiod, this method considered difficult to put animals under artificial light to decrease daylength during of anestrus period. Waller et al, (1988) found that the ewes treated orally with 2mg melatonin daily in anestrus season, have more estrus cycles than control ewes and similar to ewe treated with progesterone, and PMSG.

I.4 Induction of Ovulation

I.4.1 Ram effect

The ovulation during anestrus season does not occurs because the secretion LH is low, it not promote to development of ovarian follicles, and the corpora lutea, plasma concentrations of progesterone also remain very low (Chanvallon et al, 2008).

Introduction of ram into ewes during anestrus causes secretion of LH hormone within a few minutes, the increase levels of LH hormone that stimulates follicular growth and estrogen secretion, the increase of estrogen concentration in blood has two effect, the first 2-12 hours decrease of FSH and LH hormone ,and between the period in 12- 48 hours

it induces preovulatory surges of both LH and FSH, the high levels of LH hormone induces ovulation and formation of a CL (Martin et al ,1986). In some ewes of the first ovulation of their estrus cycle , the CL secretes little progesterone and regresses within 6 days (Martin et al ,1986), first ovulations are generally silent (without heat), second ovulation with estrus signs in 17-19 days after introduction of ram (Ungerfeld et al,2003).

The ovulation are occur within 50 hours in most ewes after introduction of ram (Martin et al, 1986, Rosa and Bryant, 2002).

However, if the ewes treated with progesterone before introduction of ram, the CL formed are normal at the first ovulation (Martin et al, 1986).

I.4.2 Nutrition

The nutrition or flushing in the sheep considerable of the factors that effect on the ovulation rate. Also, it is affected on blood concentration of minerals and hormones (Sabra and Hassan, 2008). The nutritional effects on the reproductive performance of female small ruminants classified to: (i) static effect, refers to the higher ovulation rate observed in heavy compared with light ewes. (ii) dynamic effect, refers to increase ovulation due to increase in live weight and body condition during short period (e.g. 3 weeks) before mating, that refers increase in ovulation rate. (iii) immediate nutrient effect that are short period (4-6 days) increases ovulation rate with no changes in live weight or body condition (Landau and Molle, 1997).

Immediate nutrient effect for energy are more important than protein, and the effects energy and protein can not be separated because about 35% of glucose requirement can be provided from amino acids, however, increase in protein quantity can be increase in glucose, glucose is considerable source of energy for the ovary(Landau and Molle, 1997). However, when use flushing such as lupin grains this high energy and protein increase plasma estrogen, progesterone and gonadotropin hormone as will as ovulation rate. (Sabra et al,1997, Sabra and Hassan,2008).

There studies conducted during breeding season to evaluate reproductive performance for using flushing such as Shahneh et al, (2008) he used affect of the flushing and (eCG) on reproductive traits, from during of experiment consist of four groups, which used CIDER in all groups for 18 days, the group 1 control, group2 used 600IU eCG, group 3 eCG + flushing and group 4 used flushing, found the twining rate are higher in group three, the pregnant rate was higher in group four.

Other researches such as Sabra and Hassan, (2008) used nutritional flushing for reproductive performance by use yellow corn (58%), soy bean (41%) and minerals for one month before mating, found increase of incidence estrus, lambing rate and pregnant rate than non flushing group. (Sabra and Hassan, 2008).

I.4.3 Gonadotropins

Ovulation rate in ewes is lower when induced during anestrus than during the breeding season. Ovulation rates were increased by injection of gonadotropins such as equine chorionic gonadotropin (eCG) follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) and mixed gonadotropin preparations, such as PG 600 which contains 400IU PMSG and 200IU hCG. In general, the use of gonadotropins to increase prolificacy has been hampered by great variability in the ovulatory response (Knights et al, 2003).

There are many factors effects the onset of estrus in sheep and goats in breeding and non breeding season sach as nutrition**,** stress, type of progesterone, rams effect and gonadotropin hormones (Doney et al, 1973, ,Greyling and Van Nierkerk, 1990, Mani et al, 1992, Romano, 1996, Romano, 1998, Romano, 2002).

Some researchers studied the effect of different PMSG doses on pregnancy and lambing rate in different sheep breeds. Fallah and Farzaneh, 2007 used CIDR-G and different levels of PMSG (300,400,500and600 IU) in Balouchi Ewes, found no different among groups in multiple birth ,but 400IU groups was highest in lambing rate and the twining rate was highest in 600IU group (Fallah. and Farzaneh. 2007). Others studies conducted on Awassi sheep to evaluate the effectiveness of intravaginal sponges for oestrous synchronisation and the effect of (PMSG) on twinning rate, sush as Zarkawi, (2001) used sponges contain on 60mg MAP alone and MAP

with 500IU PMSG in breeding season, he found the twining rate was increasing at used 60mg MAP with PMSG.

Others researches used 40mg FGA and different levels of PMSG (500,600,750IU) in breeding season, found pregnant rate and litter size were increasing when used 600 and 750IU PMSG. (Timurkan and Yildiz, 2005). Also, Akoz et al, (2006) used 30mg, 40mg FGA with different doses PMSG (300,500,700IU), found of use 30, 40mg FGA with 700IU PMSG was rather more effective in pregnant rate and lambing rate. (Akoz et al, 2006).

The average interval from sponge removal to onset of estrus is 32 to72 h, but the intervals estrus onset was 49.7 ± 15.7 after injection of 200 IU (Fonseca et al.2005), which considered to be sufficient to stimulate ovulation and estrus without causing multiple ovulation (Ritar, 1993, Fonseca et al.2005).

Chapter three
Material and Method

II.1 Animals:

Twenty Assaf ewes were used in this study, the ewes were 3 - 5 years old, 65 - 70 kg weight and four healthy rams (Assaf) aged 3 - 4 years weighing 90 – 100kg. This study was carried out in special farm owned by Gazal family at Sabastia village near Nablus city. All ewes had previously lambed and weaned their last lamb.

Estrous induction and synchronization was conducted during out of the natural breeding season. The animals were kept indoor at night and had access to natural grazing area for most of the day. Indoor ewes were offered diets of concentrated feed with wheat straw. Water and mineral licks were available *ad libitum*.

II.2 Experimental Designs:

Ewes were distributed randomly in a factorial design. The ewes distributed into 4 groups with 5 ewes in each, as the following:

Group I:

Ewes in this group received hormonal treatments comprised of intravaginal sponges impregnated with medroxyprogesterone acetate (MAP) with a 60 mg dose (Syncro-Breed, The Advanced Veterinary Manufacturing Co. Ltd/ Palestine) sponges were inserted for 14 days plus an intramuscular injection of 300 I.U of PMSG (AVCP Manufacturing Co. - Ramallah - Palestine) at time of sponge removal.

Group II:

Ewes in this group received the same progesterone treatment as Group I, but instead of 300 I.U of PMSG they were injected by 600 I.U of this hormone at sponge removal.

Group III:

Ewes in this group received the same type of sponges used in all groups, but sponges were replaced by a new on at the 7th day of treatment. The new inserted sponge were removed after 7 days and the ewes injected of 300 I.U PMSG at sponge removal.

Group IV:

Ewes in this group received the same progesterone treatment as group III but the PMSG dosage was as in the group II (600 I.U).

The four groups are summarized in table (1).

Table 1. Treatment groups

Treatment Groups	**Progesterone Treatment**	**PMSG Treatment**
Group I	60mg MAP for 14 days	300 I.U PMSG
Group II	60mg MAP for 14 days	600 I.U PMSG
Group III	1st 60mg MAP sponge for 7 days 2nd 60mg MAP sponge for another 7 days	300 I.U PMSG
Group IV	1st 60mg MAP sponge for 7 days 2nd60mg MAP sponge for another 7 days	600 I.U PMSG

At the day of sponge removal and PMSG injection, 4 teaser rams were allowed to run with the treated ewes. Estrus was monitored every 6 hours for 3 days.

The ewes considered in estrous when they were mounted by the teaser rams. Ewes that detected by teaser rams have been introduced to the rams to be bred.

II.3 Blood Sampling and Hormonal Assay:

Blood samples were collected immediately before sponge insertion, however, blood were collected at day 7 following sponge insertion and every 5 days starting from the day of PMSG injection for 15 days to compare P4 concentrations among groups for estrus detection and pregnancy diagnosis and determination of animal reproductive state.

All blood samples were collected via jugular vein puncture into blank tubes. Tubes were kept at room temperature for serum harvesting. Harvested serum was pipetted into plastic vials and were stored at -20˚C until hormonal assaying. The progesterone analysis procedure are shown in appendices (1)

Following sponge removal some reproductive parameters were recorded and evaluated for each animal of the four groups.

These parameters are:

1. Incidence of Estrus: the number of ewes detected by rams within 72 hours following sponge removal.

2. Onset of Estrus: estrus occurring within 120 hours following sponge removal.
3. Duration of Estrus: estrus duration from onset until the ewes become not receptive to rams for mating.
4. Pregnancy Rates: number of pregnant ewes/number of ewes showing estrus and mated in each group. (Pregnancy was diagnosed by detecting the levels of P4 at day 15 after sponge removal and by ultrasonography by the day 50 post mating.
5. Lambing Rate: number of ewes lambing/number of pregnant ewes in each group.
6. Litter Size: the number of total lambs/number of lambing ewes.

II.4 Statistical Analysis:

The data were analyzed by using the SPSS (Statistical Package for the Social Sciences 16) using one way ANOVA.

Progesterone concentration in relation to single or twins births were analyzed using the SPSS using regression and correlation model.

Chapter four
Results and Discussion

III.1 Response to Estrus induction:

From the 20 Assaf ewes used in the trial, 16 ewes exhibited signs of estrus during the 120 hour observation period, which group one and group two are 80% and group three and group four are (60%, 100%) are shown in table (2). The percentage of ewes exhibiting estrus in this trial was comparable to values reported in the literature (Simonetti et al., 2000, Dogan, 2006). While Dogan, (2006) reported 88,9% estrus response by using60 mg of MAP and 500 IU of PMSG during non- breeding season, 80.87% estrus response have been obtained by Simonetti et al, 2000 who used 60 mg MAP. Higher estrus responses (100%) have been reported by Hashemi, et al (2006) in studies involving the use 60 mg of MAP and 500 PMSG in Karakul ewes, outside the breeding season. The slight differences between the results obtained in the current study and those of the proceed researchers may be due to differences in the breed of sheep used and the seasons in which the studies were executed.

III.2 Onset of Estrus:

The time from sponge withdrawal to estrous and the duration of the induced estrus period following the two methods of sponge treatment for the two different PMSG doses are shown in Table 2 and a summary of the distribution of animals showing estrus is set out in Table 3.

Table (2) Mean estrus responses following estrous synchronization with The aid of different Progesterone protocols and different PMSG Doses.

Treatment	Groups		n	Estrus response (%)	Onset of estrus (h)	Duration of estrus (h)
One sponge method (14days)	Group I	300 I.U PMSG	5	4 (80)	60.7 ±20.3	26.4±4
	GroupII	600 I.U PMSG	5	4 (80)	51.3±25.2	12.1±7.3
Two sponge method Each(7days)	Group III	300 I.U PMSG	5	3 (60)	52.6±10.9	12.8±8.3
	Group IV	600 I.U PMSG	5	5 (100)	46.8±14.3	13.7±11.3

The time from sponge withdrawal to the onset of estrus was not significantly different between the two progesterone application methods or the two PMSG doses.

The time to estrus was slightly longer (60.7h) in the groups treated with one sponge for 14 days and 300 I.U PMSG at sponge withdrawal compared to the other groups (51.3, 52.6, and 46.8).

Absence of significant differences in terms of time to the onset between ewes treated with the two different intravaginal sponges demonstrate a similar efficiency of the two synchronization methods in inducing estrus

during the non-breeding season in the Assaf ewes. So using one sponge as in the group one and two is less costly than the other two methods used in the other groups. The time of estrus onset results (46.8 – 60.7) of the current trial are in agreement with the previous findings of Greyling and Van der Nest,(2000) the time of onset (50.7±26.3) and with Dogan, (2006) 30 and 60 hour. To the contrary, Amer and Hazzaa (2009) noted the time from sponge withdrawal to the onset of estrus to be later in ewes treated with FGA for 12 days. Simonetti et al, (2000) recorded estrus to occur 55.94, 56.74 and 57.7 hours after using sponges impregnated with 40,50,60 mg progesterone respectively.

Comparing the results of this study to the results obtained by AKÖZ et al, (2006) who used different concentrations of progesterone in sponges, it is cleared that there is no significant differences between the using of two sponges or different progesterone levels in the sponge.

As mentioned in the table (3), it is found that using of two sponges may accelerate the estrus induction after sponge withdrawal, Two of the ewes treated with one sponge showed estrus signs after 73 hours of sponge withdrawal.

Table (3) the distribution of the estrus response at different time intervals among treatment groups.

Treatment		**n**	**Time intervals (h)**				
			0-24	**25-48**	**49-72**	**73-96**	**97-120**
One sponge	**300 I.U PMSG**	5	0	1	2	1	0
	600 I.U PMSG	5	0	2	1	1	0
	Total	10	0	3	3	2	0
Two sponge	**300 I.U PMSG**	5	0	1	2	0	0
	600 I.U PMSG	5	0	2	3	0	0
	Total	10	0	3	5	0	0

III.3 Duration of Estrus:

Duration of estrus are shown table (2) was not significantly different in all treatment groups (P>0.05). However, the duration of estrus was slightly longer(26.4) in the group one sponge for 14 days and 300 I.U. PMSG compared to other groups (12.1±7.5, 12.8±8.3, 13.7±11.3h). The results of our study could be the shorter compared to previous studies (Hashemi at el, 2006) who reported that the duration of estrus were between 18 and 72 hours with an average around 36 hours and the duration of estrus to be

22.11±3.4 by using MAP with 500IU eCG out-breeding season. Dogan , (2006) reported an estrus duration of 28.5±2.2. with 60mg MAP and 500 IU PMSG.

The reason of short estrus duration period in this study and the variation in two groups 300IU PMSG and 600IU PMSG my be due to lower estrogen in blood during the non-breeding season and to breed differences, age and geographical location (Evans and Maxwell, 1986, Gordon, 1997, Hashemi et al, 2006).

III.4 Litter size:

The litter size estimated for the different levels of PMSG treatment are shown table(4). From the 16 ewes that observed in estrus, 22 lambs were obtained. Ten lambs were delivered from the 7 ewes treated with 300 I.U. PMSG. However, 12 lambs have been obtained from 9 ewes treated with 600 I.U. PMSG at sponge removal.

The mean litter size in the PMSG groups were estimated to be 1.43 for the ewes injected with 300 I.U. PMSG compared to 1.33 for the ewes injected with 600 I.U. PMSG, therefore, there was no significant difference in litter size rates between the groups. This results were in agreement with pollott et al, 2004 who reported that the mean litter size in Assaf breed to be 1.57 (Pollott and Gootwine, 2004), although increased PMSG doses should increase the number of follicular growth and so to increase twining rate and litter size, the results of the present study did not show any significant

differences between the groups treated with 300 or 600 I.U PMSG. However, in the present study there was noticeably increase in the litter size in group treated with 300 I.U, Opposing the phomena of gonadotropin effects on ovulation rate; gonadotropin hormones function to increase litter size and twining rate. Researchers reported that the using of 400 I.U PMSG increased the litter size when compared to ewes injected with 200 or 300 I.U. PMSG. (Toteda et al, 1990, Cruz et al, 1990)

Table (4) The litter size estimation for the different levels of PMSG treatment

Treatment Groups	**Number of lambing ewes**	**Number of lambs (litter size)**
300 I.U. PMSG	7	10 (1.43)
600 I.U. PMSG	9	12 (1.33)

III.5 Serum Progesterone Concentration:

Mean serum progesterone concentration (ng\ml) of ewes are shown in table (5). The mean serum progesterone concentration during the 15th day after sponge withdrawal were 7.18 ng/ml and 2.7 ng/ml in the groups treated with 600 and 300 I.U. PMSG, respectively.

Table (5) Mean serum Progesterone concentration (ng/ml)of ewes treated with 300 and 600I.U PMSG during the 15th day of synchronization

	Ewes treated with 300 I.U PMSG	**Ewes treated with 600 I.U PMSG**
Mean Progesterone concentration (ng/ml)	2.7	7.18

No significant differences were found between the mean P4 concentration when administration the different PMSG doses. This not comparable with Amer and Hazzaa, (2009) who reported that P4 concentration tend to increase gradually and reach maximum between day 11 and 20 days when PMSG administrated with high doses. And with Amiridis et al, (2002) who reported that larger dose of FSH attributed to superovulation and increase the number corpora lutea which lead to increase P4 concentration.

Proportion of ewes with one or two lambs and their serum Progesterone concentration at day 15 after sponge withdrawal are demonstrated in table (6). The number of ewes that had P4 concentration between 1 – 2 ng/ml were 7 where 4 of them delivered one lamb and 3 had delivered two lamb for each. However, the number of ewes that had P4 concentration > 2 ng/ml were 6 ewes with two lambs each Progesterone concentration between ewes gave birth to single and twins were significantly different.

Based on the number of lambs delivered and measurement of P4 concentration 15 days after sponge withdrawal a strong, positive, linear

between blood P4 concentration and the number of lambs delivered was found.

The blood P4 concentration values in ewes carrying single or twin fetuses are comparable to those reported by researchers (Ezzo and shalaby, 1990. Greyling and Van Der Nest, 2000, Amiridis et al, 2002).

According to these results measurement of P4 concentration during pregnancy can be used to diagnose pregnancy with single or multiple fetuses.

Table(6) Proportion of ewes with one or two lambs and their serum Progesterone concentration at day 15 after sponge withdrawal.

Lamb No./ewe	**Day 15 ewes with P4 concentration 1-2 ng/ml**	**Day 15 ewes with P4 concentration >2 ng/ml**
One lamb	4	0^{a}
Two lambs	3	6^{b}

Means in the same column with different letters (a and b) differ significantly ($p< 0.05$)

P4, serum progesterone concentration

Conclusion:

in conclusion, this study suggests that changing sponges or increasing the dose of PMSG is of no advantage since the half dose of PMSG has the same effects on number of ovulations and liter size, also changing the sponge did not affect the rate of synchronized ewes. Significant savings can be achieved applying 300 I.U PMSG with one sponge.

This study showed that measurement of P4 concentration is a good diagnostic tool of single or twins pregnancies. However, more research on the tested parameters is required especially when dealing with larger herds of ewes.

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Appendices

Appendices (1): Progesterone Analysis Procedure:

1. Preparation of 50 ml of stander , samples and controls.(Ovine Progesterone ELISA Test – Endocrine Technologies, INC. USA)
2. Added 100ml of progesterone enzyme solution to (standred, samples and control), Mix for 30 sec. and incubator at 37 C for 1hour.
3. Used wash solution (250-300ml) per wells 5 times.
4. 100ml added substrate solution to each wells and then incubator wells at room temperature for 20 minutes without shaking.
5. 50 ml stop solution was added to each well.
6. Used ELISA Plate Reader (Digital and analog system "DAS", Italy) to read the absorbance at 450nm and then calculate the concentration for stander, samples, control and blank and using plot a graph on semi-log graph paper.

Appendices (2): Ovine Progesterone Elisa Kit

OVINE PROGESTERONE

OVINE PROGESTERONE ELISA KIT

INTENDED USE

The Microwell Progesterone ELISA is an enzyme immunoassay system for quantitative determination of progesterone levels in serum/plasma. The test is intended for professional use as an aid in the diagnosis and monitoring of conditions related to serum progesterone in ovine and related species.

INTRODUCTION

Progesterone is a steroid hormone (C21 steroid, pregn-4-ene-3, 20 dione) and is synthesized from both tissue and circulating cholesterol. The principal production sites are the adrenals and ovaries and placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by kidneys.

The primary role is played in reproductive organs. The monitoring of LH and progesterone will help the breeders.

TEST PRINCIPLES

The progesterone quantitative Test is based on a solid-phase enzyme immunoassay based on competitive binding method. A sample (serum plasma/urine) containing an unknown amount of progesterone will compete with enzyme-conjugated progesterone for high affinity binding sites on a limited number of antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations in unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. After washing, substrate solution is added and the enzyme allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve is produced using values from standards from which absorbency values for blank tubes have been subtracted. Results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program. This kit is suitable for the direct measurement of progesterone in serum/plasma samples. It may also be used following an extraction procedure, for assaying urinary progesterone.

MATERIALS PROVIDED
1. Wells coated with progesterone antibody , 96 wells
2. Enzyme Conjugate, 12mL
3. Progesterone Standard Set: 0, 1.0, 2.5, 5.0, 10, 30ng/mL
QC1 (<2.5ng/mL) QC2 (10ng/mL)
4. Sample/standard diluent 20mL
5. TMB Color Reagent, 12 mL
6. Stop Solution (2N HCL), 6mL.
7. 20 X Wash Buffer, 20mL.
8. Instructions

Materials Required, But Not Provided	
1.	Semiautomatic pipettes: 20ul and 200ul
2.	Disposable pipette tips
3.	Microtiter plate shaker
4.	Microtiter well reader.
5.	Plate washer
6.	Absorbant paper
7.	37 C incubator
8.	Parafilm to cover plate
9.	Distilled water

PRECAUTIONS

1. CAUTION: This kit contains reagents manufactured from blood components and all blood products and samples should be considered potentially infectious and handling should be in accordance with the procedures defined by an appropriate your biohazard safety guideline or regulations.
2. The contents of this kit, and their residues, must not come into contact ruminating animals.
3. Avoid contact with the Stopping Reagent. It may cause skin irritation and burns.
4. Do not use reagents after expiration date.
5. Do not mix or use components from the kits with different lot numbers.
6. Replace caps on reagents immediately. Do not switch caps.
7. Reagents contain sodium azide (NaN3) as a preservative.
   On disposal, flush with a large volume of water to prevent azide build-up.
8. Do not pipette reagents by mouth.
9. Do not use reagents from other kits or mix with other manufactured test kits.

STORAGE & STABILITY CONDITIONS

1. Store the kit at 2-8°C upon receipt and when it is not in use. **Do not Freeze.**
2. Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
3. Allow all the reagents to reach to room temperature before setting up the assay.
4. Remove only desired number of wells and seal the bag and store at 2-8°C as before.
5. Do not at any time mix or use components with other manufacturer kits. Do not use the kit components after expiration date.

INSTRUMENTATION

A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for use in absorbency measurement

SPECIMEN COLLECTION AND PREPARATION

1. This kit is suitable for use with serum or heparin plasma samples. The use of hemolytic or lipemic samples will affect results and also samples with bilirubin may interfere with the assay.
2. No special preparation of the samples is required. A venous blood sample (enough to produce about 0.5 ml serum) is collected aseptically.
3. If the sample is not tested immediately refrigerate at 2-8°C. If the storage period greater than 3 days are anticipated, the specimen should be frozen and repeated thawing and freezing should be avoided.
4. If the sample is turbid or contain precipitate may give false results. Such samples should be centrifuged before use. Serum samples with gross lipemia, hemolysis and turbidity should not be used.

REAGENT PREPARATION

1. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 2-8 C for couple of weeks.
2. Dilute highly concentrated specimen samples with dilution buffer and mix well before use in the assay.

3. Standard solutions if not used for more than a week, should be kept frozen at -20C.

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 ul of standards, samples, and controls into appropriate wells within 5 minutes.
3. Add 100ul of progesterone Enzyme Conjugate Solution to each well. Mix well for 30 sec. and incubate at room temperature (~25C) for 60 minutes. It may be consistent to incubate at 37C. for 1 hour. You may use parafilm to cover the wells or use appropriate zip-lock bag to store the plate during the incubation.
4. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
5. Add 100 ul Substrate solution to all wells. Remember to follow the pipetting order.
6. Incubate the plate at room temperature (18-28°C) for 20 minutes without shaking.
7. Stop reaction by adding 50ul of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed.
8. Read the absorbency at 450 nm with a microwell reader.

NOTE: The substrate incubation should be carried out within the temperature range 20-25C. For temperature outside this range, the duration of the incubation should be adjusted.

CALCULATIONS

1. Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
2. Subtract the value for blanks from those for standards, control and unknown samples.
3. Calculate the B/B)% values by dividing each value by the value for the zero-standard.
4. For the standards, plot a graph on semi-log graph paper with B/BO% values on the ordinate and the progesterone concentrations (ng/ml.) on the abscissa.
5. Using the graph read off the progesterone concentrations for the unknown samples.
6. The values above the readable and below the readable range should be repeated using appropriate dilution.

QUALITY CONTROL

The sensitivity of the assay is 1 ng/mL and each laboratory should establish its own base levels based on the ovine species and physiological situation.

Good Laboratory practice requires that quality control specimens be run with each standard curve to establish assay performance characteristics such as recovery, linearity, precision and specificity.

LIMITATIONS OF THE TEST

1. The present Endocrine's ELISA system designed here is for estimation of progesterone levels in serum/plasma samples by a professional only.
2. The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions.
3. The results obtained with this assay should only be used as an adjunct to other diagnostic procedures and information available to the experts in the field.
4. Trained and skilled professional only should perform the assay.

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تحفيز الشبق بطرق مختلفة لدى أغنام العساف خارج موسم التناسل

إعداد

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اشراف

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الملخص

أجريت هذه التجربة على عدد من الأغنام العساف عددها 20 وذلك لتحديد تأثير مصل فرس الحامل (PMSG) بمستويات مختلفة (300 IU, 600 IU)، واستخدام طرق مختلفة من الاسفنجات المشبعة بهرمون البروجيستيرون (اسفنجة واحده ل 14 يوم إسفنجتان كل منها لمدة 7 أيام) على الاستجابة للشياع وفترة بدأ الشياع وفترة الشياع ونسبة التوائم وتركيز هرمون البروجيستيرون في الدم خلال فترة الشياع وخلال فترة الحمل المبكر.

في شهر ابريل والذي يعتبر الموسم الغير الطبيعي للتناسل للنعاج في فلسطين, حيث تم تقسيم الأغنام إلى أربعة مجموعات تحتوي كل مجموعة على 5 نعاج من العساف و وتم وضع الاسفنجات المهبلية التي تحتوي على 60 ملغم من Medroxyprogestron acetate (MPA) ل 10 أغنام من العساف وبعد 7 أيام من إدخال الاسفنجة تم سحب الاسفنجة وإدخال اسفنجة جديدة في المجموعتين الثالثة الرابعة، أما العشرة أغنام الأخرى فقد وضعت الاسفنجات لمدة 14 يوم متتالية في المجموعتين الأولى و الثانية, وفي اليوم 14 تم سحب كل الاسفنجات وحقن المجموعات الأربعة ب IU300 أو IU600 من PMSG في العضل. بالرغم من ظهور بعض النتائج المتفاوتة لم يكن

هناك أي اختلافات معنوية من طريقي تطبيق البروجيستيرون المختلفتين أو الاختلافات في الجرعات المختلفة من PMSG.

وبناءا على النتائج الواردة في الدراسة الحالية، فأن استعمال اسفنجة واحدة متبوعة بجرعة عضلية مقدارها (IU300) من هرمون مصل الفرس الحامل تعتبر كافية لتحفيز الشبق خارج الموسم التناسلي، بالإضافة إلى كونها اقل كلفة من استعمال جرع عالية من الهرمون. ايضا و من خلال فحص نسبة هرمون البروجسترون في الدم في اليوم الخامس عشر من الحمل فأنه من الممكن تشخيص الحمل إذا كان الحيوان حامل بجنين واحد أو أكثر.

جامعة النجاح الوطنية
عمادة كلية الدراسات العليا

تحفيز الشبق بطرق مختلفة لدى أغنام العساف خارج موسم التناسل

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قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في الإنتاج الحيواني , عمادة كلية الدراسات العليا, جامعة النجاح الوطنية, نابلس , فلسطين

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