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Embryo Transfer In Farm Animals

Current Ovine Embryo Transfer Technique

A Research
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بسم الله الرحمن الرحيم

(فَتَعالى اللهُ المَلكُ الحَقُ ولا تعجَلْ بالقُرانِ مِنْ
قَبْلِ أنْ يُقضَى إليكَ وَحُيه وَقل رَبِ زدْني عِلْماً)

صدق الله العلي العظيم

سورة طه (أية 14)

Certificate of Supervisor

I certify that the research entitle(**EMBRYO TRASFER IN THE FARMS ANIMAL Current Ovine Embryo Transfer Technique**) was prepared under my Supervision at the college of Veterinary Medicine / University of Al-Qadissiya .

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21. 4, 2016

Dedication:

To .. my God and Imam

To .. my Parents

To .. my Teacher

To .. my Friend and all Family

Ahmed Maluki

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I Thank God for his tender.. Thank truthful on their promise.. I Thank my parents on their virtue .Thank teachers on their giving, especially dr. Khalid Mohammed Karam and dr. Abbas Fadhil Daham .. Thank all the good friend especially Karrar Yasser and Jaafar Kadhim. Finally, Thank for myself on patience.

AHMED SALAH

Summary

Summary

The Embryology of modern science that uses genetic improvement in animals and has an important role in the economies of Animal Production. The aim of embryo transfer in sheep and goats to conduct genetic improvement and speed up the program to create high herds production (production of twins - a high production of milk) in a short period of time, through the transfer of embryos from mothers (ewes) with excellent qualities and hereditary in their production (called ewe giving Donors) to the mothers (ewes) Other average production called futuristic ewes Recipients. And it is performed embryo transfer program through the work of the so-called multiple ovulation Superovulation a method used to increase the number of embryos obtained from a single sheep (and female animals in general). There are several successful ways to multiple ovulation. Although the first successful embryo transfer was in rabbits in 1890, but the first process for the transfer and implantation embryos in farm animals were in sheep in 1949 and was the first successful embryo transfer in cattle in 1951 in addition to the first commercial company formed for the implementation of the transfer program embryos in animals (cows) in 1971. And, for information, the first birth of a child after the transfer of human embryos in 1978 was.

Embryo Transfer In Farm Animals

Current Ovine Embryo Transfer Technique

Contents

LIST OF CONTENTS

CHAPTER ONE

Introduction

1.Introduction

1.1. Introducing embryo transfer

Embryo transfer (ET) is a technique by which fertilized ova are collected from a genetically outstanding dam (donor) and transferred to genetically less distinguished females (recipients) to serve as surrogate mothers for the remainder of pregnancy.

Hormonal treatments to induce multiple ovulation and ET enable the intensive use of genetically superior females. Over the last 25 years, considerable progress in ET has been achieved, improving the genetic quality of sheep and goats. This has required maximizing embryo production and survival to obtain offspring of high genetic value. It must be emphasized that a genetically superior mother can be used in ET programs more than once, thus multiplying her reproductive potential by using genetically inferior animals as recipients of genetically superior embryos (Mueller 1993).

The natural reproductive potential of each species and breed is a limiting factor for the diffusion of genetic improvements. Under traditional sheep and goat breeding conditions, the number of offspring from a female per year is one or two. Therefore, during the female reproductive lifetime, six to eight offspring can be obtained. Embryo transfer increases the reproductive potential of high genetic value females by taking advantage of the great oocyte reserve in the ovary. Hormonal stimulation of the ovaries induces multiple ovulations, resulting in an increase in the average ovulation rate of a given breed. Consequently, a considerable number of offspring can be obtained in a short time.

Embryo transfer shortens the generational interval, resulting in greater genetic progress. At the same time, artificial insemination and ET together constitute excellent tools for genetically improving flocks and herds isolated from suppliers of male improvers.

1.2. Historical review on embryo transfer in farm animals

The first successful transfer of fertilized ovum was carried out in rabbits by Heape (1890) in Great Britain, while the first embryo transfer in domestic animals was reported in sheep and goats by Warwick and Berry (1949).

In 1951, the first calf was born via the surgical technique of embryo transfer by Willett et al.1955 and during the 1960's, surgical technique of embryo transfer had been established as a research tool in cattle, sheep and goats. After then in the early 1970's, embryo transfer has been applied extensively in cattle consequently the technology has advanced most rapidly in this species.

With the advent of efficient non-surgical techniques for the recovery and transfer of bovine embryos, as well as effective methods for preserving embryos in liquid nitrogen, demand for embryo transfer services increased dramatically in both dairy and beef industries.

The first successful equine embryo transfer was reported in UK by Allen and Rowson (1975). In 1983, the first water buffalo calve was born by embryo transfer in U.S.A by Drost et al.(1983), on the other hand the first successful non-surgical embryo transfer in camelidae with birth of live offspring was reported in the llama by Wiepz and Chapman (1985). Over the last 20 years, embryo transfer has been used in Australia and New Zealand to incorporate Angora goat genetic material with little health risk.

In France, a program for genetically improving dairy sheep is underway (the Lacaune breed, used in the manufacture of Roquefort cheese). International trade in frozen sheep and goat embryos has led to the worldwide diffusion of germplasm, with very low health risks. As a consequence, there have been rapid genetic improvements in different breeds and the establishment of alternate meat, milk, wool and hair production (e.g. mohair or cashmere) choices.

1.3. Embryo transfer in sheep and goats

The first embryo transfers in sheep and goats were carried out 75 years ago (Warwick et al.1934). After the 1960s, they were continued in Australia (Moore and Rowson 1960) and in New Zealand (Tervit and Havick 1976), which helped to clarify the conditions and possibilities of this biotechnology. The natural reproductive potential of each species and breed is a limiting factor for the diffusion of genetic improvements.

Under traditional sheep and goat breeding conditions, the number of offspring from a female per year is one or two. Therefore, during the female reproductive lifetime, six to eight offspring can be obtained. Embryo transfer increases the reproductive potential of high genetic value females by taking advantage of the great oocyte reserve in the ovary.

Hormonal stimulation of the ovaries induces multiple ovulations, resulting in an increase in the average ovulation rate of a given breed. Consequently, a considerable number of offspring can be obtained in a short time. Embryo transfer shortens the generational interval, resulting in greater genetic progress. At the same time, artificial insemination and ET together constitute excellent suppliers of male improvers.

CHAPTER TWO

Applications of embryo transfer

2. Applications of embryo transfer

Embryo transfer is a method of assisted reproduction based on producing multiple embryos in a female donor (genetically superior mother) that are then transferred to various female recipients (gestating mothers).
Hormonal treatments to induce multiple ovulation and ET enable the intensive use of genetically superior females. Over the last 25 years, considerable progress in ET has been achieved, improving the genetic quality of sheep and goats. This has required maximizing embryo production and survival to obtain offspring of high genetic value. It must be emphasized that a genetically superior mother can be used in ET programs more than once, thus multiplying her reproductive potential by using genetically inferior animals as recipients of genetically superior embryos (Mueller 1993).

Therefore application of embryo transfer in livestock can be detailed briefly as follows:

1- Obtaining a large number of offspring from a single donor per year. Approximately 5-12 lambs can be produced annually from each donor ewe.

This large number results in:

a- Rapidly increasing the rare blood lines.

b- selecting the desirable genotypes

In the middle east, several successful trials have been performed by the authors and their colleagues to produce local desirable breeds of sheep from exotic breeds. Naimi (Awassi fat-tailed) lambs were obtained from transferring Naimi embryos to Marino and Dorper (long-tailed) recipients.

c- Accelerating the genetic improvement by facilitating progress testing of females and thus reducing the generation interval.

2- Controlling disease transmission zona pellucida (a gelatin- like shell surrounding the embryonic cells) of the embryo appears to be an effective barrier to infection of the embryonic cells from the uterine environment.

It has been postulated that washing or treating embryos with trypsin in vitro removes any bacterial or viral contamination from zona pellucida. Accordingly, embryos transferred from donors infected with enzootic diseases.

3- Import and export of efficient methods for cryopreservation of embryos provides the following advantages:

a- Possibility of transferring an entire herd in the form of frozen embryos in small liquid nitrogen container. This will greatly reduces the expenses of transport. It has been estimated that the expenses of the international movement of a herd-frozen is less than the price of single live animal.

b- Importing frozen embryos prevents the risk of introduction of new diseases.

c - An additional benefit of the export of embryos over that of live animals includes a wider genetic base to select, from which particularly from exporting countries, which retain the genetic materials.

d- An important advantage of embryo transfer lies in the fact that in countries with tropical and subtropical climates the lamb resulting from an imported embryo transferred into an indigenous recipient acquires colostral immunity to local diseases in addition to adoption of the fetus to the surrounding climate. This gives better survival chance than an animal imported on the hoof.

4- Circumvention of infertility in some infertile donors

a- Various types of infertility can be successfully treated using embryo transfer method. These infertility types include uterine infection, repeat breeder, cystic ovarian diseases and adhesion of the upper reproductive tract.

b- Old donors may become repeat breeders due to the incapability of the uterus to maintain pregnancy. However they produce ovum, which are functioning, therefore embryos can be recovered from valuable old donors and transferred to suitable recipients.

c- Females suffering from blocked oviduct can be used as recipients.

5- Induction of twinning :

Embryo transfer has been routinely used in sheep and goats to increase the number of offspring per delivery researchers succeeded in getting five lambs

per delivery from Naimi sheep (which usually produce one or two lambs per delivery) as well as getting five lambs per delivery from Romanove sheep by embryo transfer.

In the cattle, the genetic selection for twinning has been largely unsuccessful. Similarly, gonadotrophin treatments for induction of twinning have been unreliable too. However, it has been estimated that embryo transfer provides a real alternative in the production of twins. Twining can be induced by:

_ Transferring of two embryos.

_ Transferring of one embryo to a previously inseminated recipient.

6- Valuable research tool:

a. Embryo transfer technology has been used exclusively studies of uterine capacity, uterine environment, maternal recognition of pregnancy, embryo uterine relations and endocrinology of pregnancy.

b. Through embryo transfer researches much useful information has been developed. For example, research indicates that embryos from heat stressed cows are severely damaged and usually die during the first few days. This is applied by collecting embryos during the cooler seasons. Freezing and transferring them to recipients during the summer months. Pregnancy rates may be doubled or tripled if compared to those expected from artificial insemination under similar conditions.

c. Embryos produce hundreds of substances as they develop. Therefore, after understanding the nature of these substances, new pregnancy tests can be developed from body fluids of the dam, such as milk, blood, urine or saliva. These pregnancy tests might even work few days post insemination.

7-Related advanced techniques.

Embryo transfer technology is considered a prerequisite for the following techniques:

a. Embryo splitting
b. Embryo sexing.
c. In vitro fertilization (IVF).
d. Intra-cytoplasmic sperm injection (ICSI) .
e . Cloning.
f. Genetic engineering.

CHAPTER THREE

Materials and methods for ovine ET

3. Materials and methods for ovine ET

3.1.Management of donors

3.1.1. Donor selection

Usually the owner selects his donor animals on the basis of performance and genetic merit. The donor must be non pregnant, either parous or nalliparous female. In addition to the genetic merit, the donor should be characterized by:

1- A good body condition and preferably gaining weight.
2- Free from diseases.
3- Successfully completed the postpartum period.
4- Regular cycling.
5- No history of reproductive problems.
6- Vaccinated against the enzootic infectious diseases.

3.1.2. Donor super stimulation and breeding

Superovulation is done in the same method of synchronizing estrus method both donor and recipient ewes can be induced by intra-vaginal sponge containing progesterone for 12-14 days in sheep and 14-18 days in goats. High dose of FSH or eCG is given only to the donor for super stimulation is given 24-48 hours before or at the time of sponge withdrawal (Armstrong et al.1983). Estrus occurs 24-36 hours after the sponge removal (Walker et al.1986) as shown in figure1.

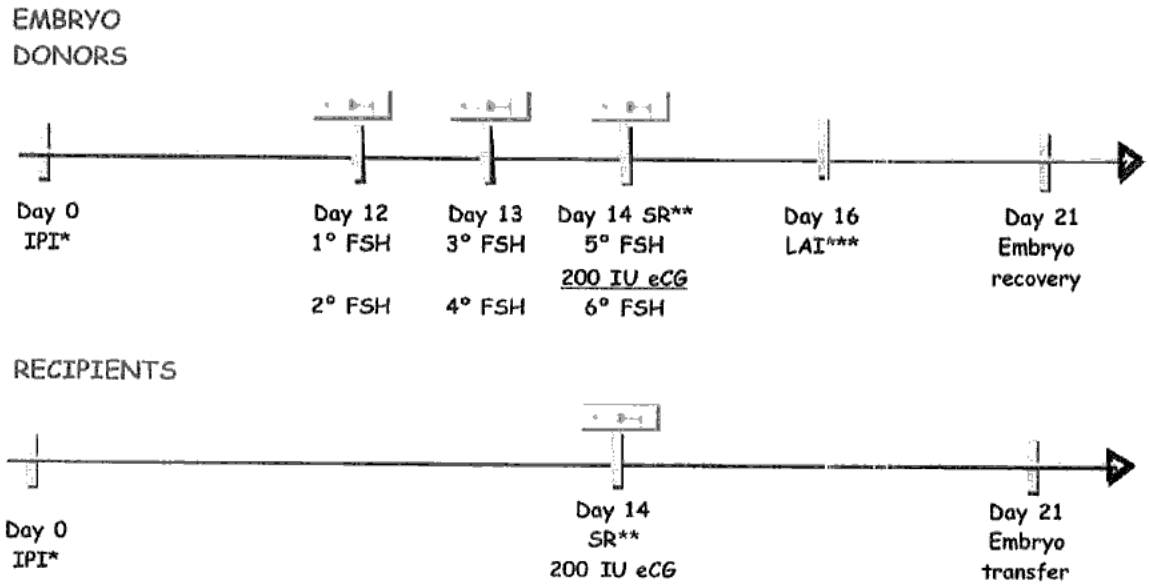


Figure 1. Hormonal treatment schedule for superovulation in ewe donors and embryo recipients.

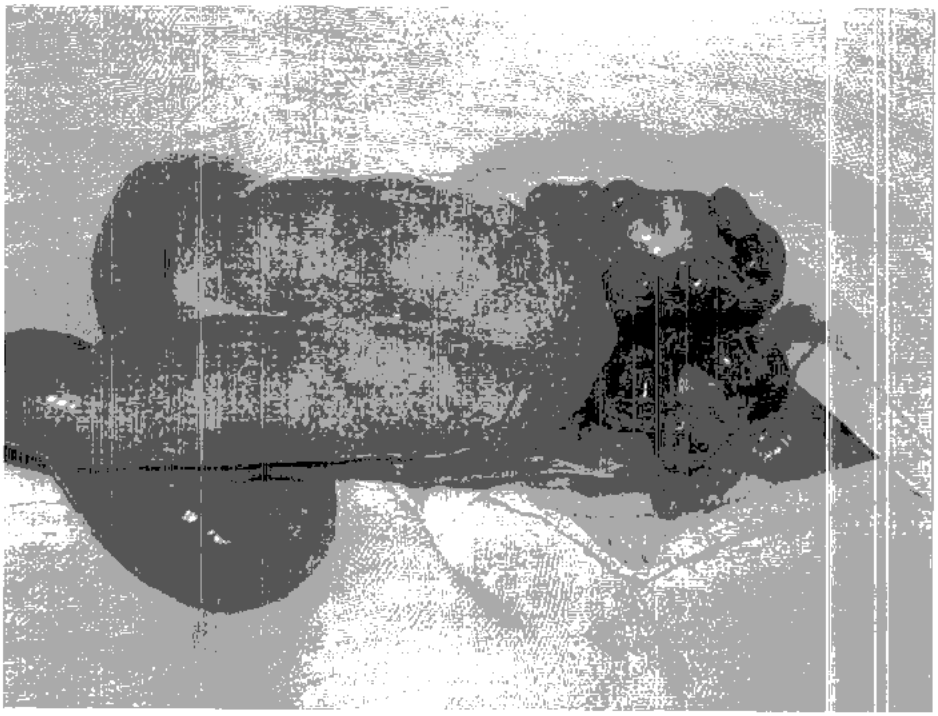


Photo 1. Ovaries with multiple ovulation after treatment with 80 mg FSH and 200 IU PMSG.

Donor ewes are bred naturally or serviced artificially using fresh collected semen at estrus (Brebion et al. 1992).

3.1.3. Embryo recovery

Surgical method of embryo recovery is still the most common technique used in sheep and goats. However, two promising techniques are now developing: the laparoscopical and the transervical methods (McKelvey et al. 1986).

There are two methods of surgical recovery; the uterine and the oviductal flush. When flushing the oviduct, the medium is passed from the uterine lumen through the uterotubal junction and along the oviduct before it is collected into a dish through a cannula.

The oviductal approach is appropriate for all stages of embryo development up to day 7.when flushing the uterus only; a medium is introduced into the uterine lumen near the uterotubal junction and recovered through a catheter inserted into the uterus near the external bifurcation. The uterine procedure is successful only with embryos recovered on day 4 or later, by which time the embryos have passed into the uterus. In sheep, embryo recovery is carried out on days 7 or 8 after sponge's withdrawal. As initial embryo development in goats is delayed 12 to 24 hours, embryos are collected on days 8 or 9 following sponge removal (Table 3). Flushing the uterus is more common and is described as follows:

1- Food and water are withheld 24 hours before the scheduled surgery. The donor is sedated by 0.3 ml setone (Xylazine) injected intravenously.
2- The donor ewe is placed on the back on the operating table and its limbs are tied to the table. The table is constructed to be inclined at an angle of about 30 degrees, so that the hind quarter is upwards and the head is downwards.
3- The abdominal area in front of the mammary gland is clipped, shaved, washed and disinfected in preparation for surgery.

4- The mammary vein is held to one side and the abdomen is opened by midline incision some 12 cm long. The incision begins as close to the mammary gland as possible to facilitate the withdrawal of uterus.

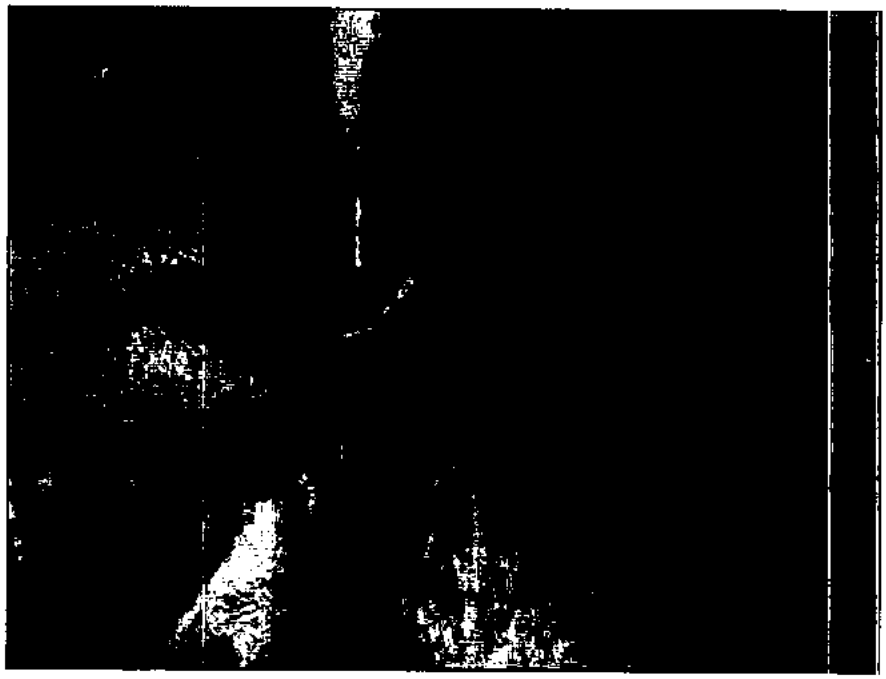
5- The forefingers of the right hand are inserted into the abdominal cavity and directed downwards and backwards to locate the uterus which is recognized by its firm horns and its pink color. The uterus is withdrawn through the incision. The number of corpora lutea in each ovary is counted.

6- All preparations and fluids should be sterile and maintained at 39 °C until use.

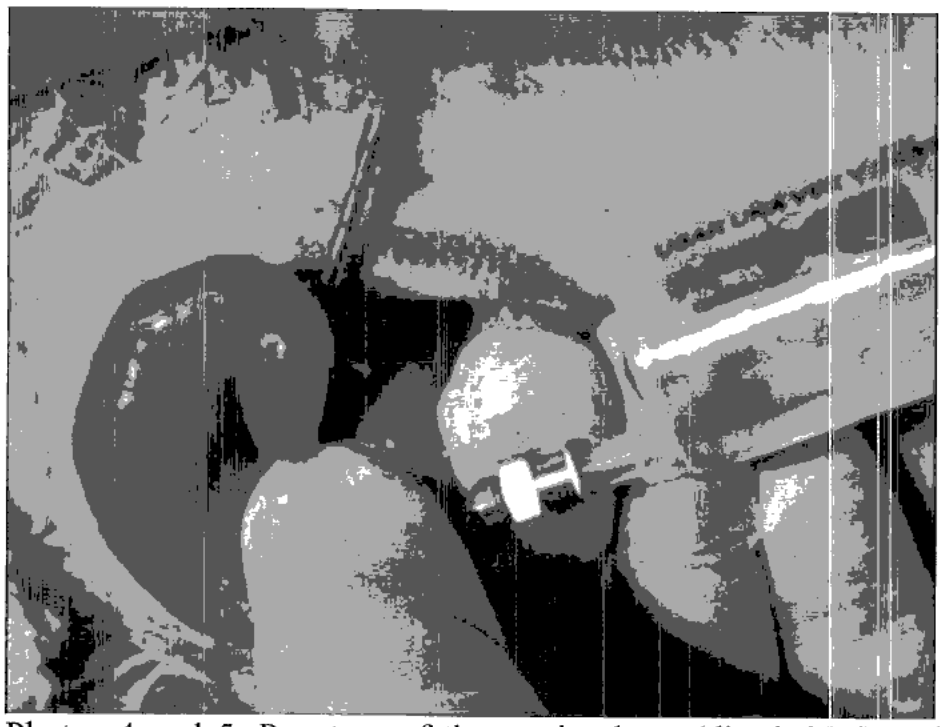
7- A blunt perforation is made by forcing the tips of artery forceps through the uterine wall just above the external bifurcation. An 8 or 10 catheter is inserted into the uterine lumen through the blunt perforation for a distance of 3-5 cm towards the oviduct before inflating the balloon with air.

8- The balloon should be sufficiently firm to prevent the movement when the catheter is pulled gently as shown in photo 2 and 3.

9- The flushing medium is introduced into the uterine lumen to rinse the uterine horn, through the blunt needle inserted through the uterotubal junction and connected with a 50 ml syringe as shown in photo 4 and 5



Photos 2 and 3. Puncture and fixing of the catheter to the uterine horn (utero-tubal junction) for surgical embryo recovery.



Photos 4 and 5. Puncture of the uterine horn (distal third) and embryo recovery by current flow.

10-Each uterine horn is rinsed with 40 ml of flushing medium (PBS) phosphate buffer solution and collected again through the embryo catheter into a searching grid dish. Efficient recovery of embryos is assumed to depend upon a free flow medium, which is able to rinse the uterine lumen thoroughly and this is achieved by a gentle massage of the uterine horn.

11-Finally the hole made to allow access for the Foley catheter is closed with a catgut suture.

Photo 6. Recovery of embryos by current flow in the Erlenmeyer collector.

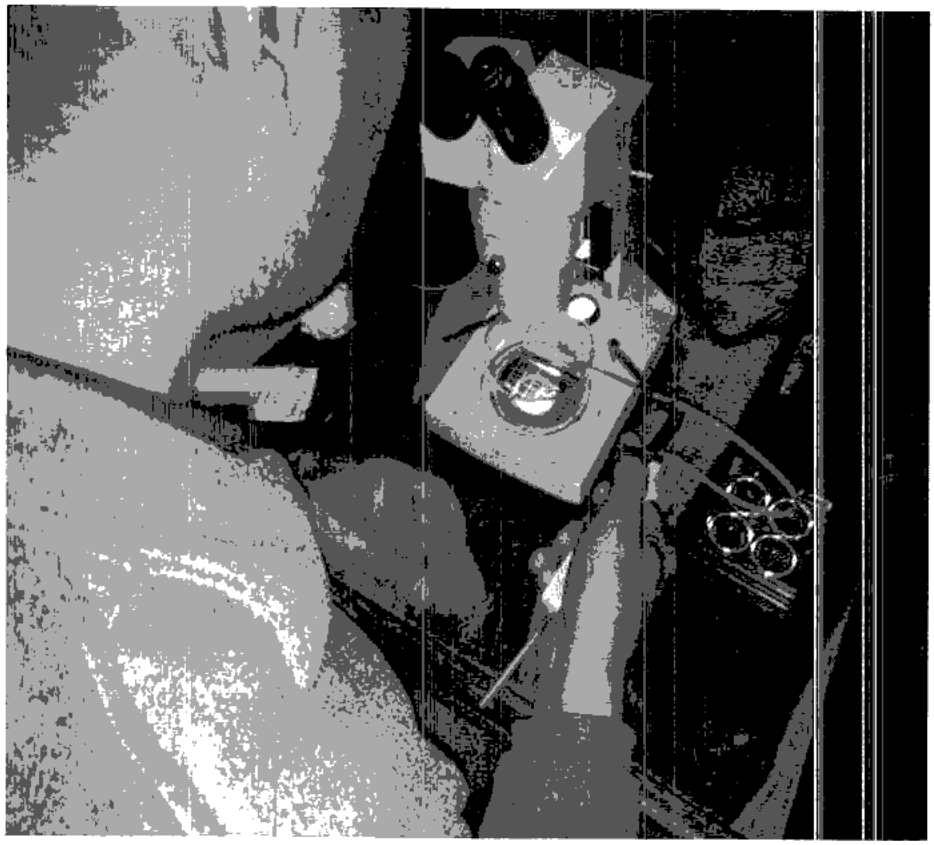
Laparoscopic and transcervical methods are recent promising methods for embryo recovery without exteriorization of the reproductive tract. However, the embryo recovery rate by these methods is still greatly lower than by the surgical methods.

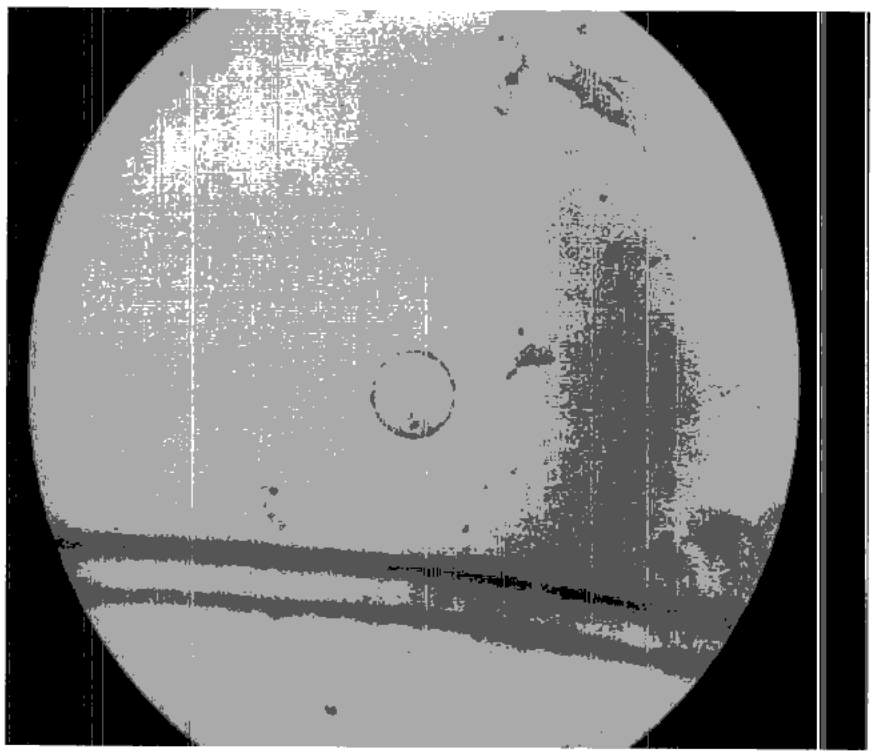
3.1.4. Identifications of embryos

The collected medium is poured into petri dishes and embryo identification is carried out under magnification and on a thermal plate at 38°C (Photo 8). A second inspection of the petri dish is always recommended. As they are identified, embryos are aspirated with a micropipette and placed in a small petri dish containing a preserving medium enriched with 20% serum, protected from light exposure and at laboratory temperature. Once the identification is finished, assessment of embryos is carried out. If possible, use a fume hood and filtered air. It is important to remember that these activities must be performed under strictly sterile conditions (Bari et al. 2003).

3.1.5. Assessment of embryo quality

Embryo assessment is carried out based on morphological aspects and under a magnification of 10 to 40x. To observe the embryos from different angles, they can be moved using a micropipette or fine pipette. The integrity of the zona pellucida and its sphericity must be observed. Embryo development must correspond to that determined by its date of collection. A 24 hours delay is tolerable. Cells must be clear and present regular boundaries; opacity, if present, indicates degeneration. Embryo survival rate is not affected by day of embryo recovery (day 5 or 6 post estrus). Embryos that are at the normal stage of development for the collection day (day 5, morula; day 6, blastocyst), show similar survival rates (74%) (Bari et al. 2003). However blastocysts collected on day 5 show a high survival rate when compared with retarded morula collected on day 6 as shown in photo 7 and 8.





Photos 7and 8. Showing the assessment of embryo quality in ewe's embryo at day 6.

CHAPTER FOUR

Recipient Management

4. Recipient Management

4.1.Estrous synchronization between donor and recipient

Synchronization of estrus in recipients by means of progestagen treatment is performed at the same time as in donors. This is to ensure that both recipients and donors reach the same day of the estrous cycle at the time of embryo recovery and transfer.

Synthetic progesterone analogs, FGA (fluorogestone acetate) and MAP (medroxyprogesterone acetate), are usually used in pessaries (intravaginal sponges) for estrous synchronization. In sheep, 14 days treatment is required; in goats, progestagen treatment lasts 17 days. In Australia and New Zealand, use of progesterone administered vaginally using controlled internal drug release (CIDR) devices is very common.

Pregnant mare serum gonadotropin (PMSG) or equine chorionic gonadotropin (eCG) has FSH activity and to a lesser extent, LH activity. It is used at the end of the progestagen treatment to synchronize estrus. In ovine and caprine species, PMSG administration at sponge removal is recommended. PMSG dose varies according to breed and reproductive physiologic condition (indicative values: 200 to 400 IU).

4.2.Embryo transfer

Embryo transfer must take place immediately after collection and, in no case, must embryos be more than two hours in the preserving medium.

Embryo transfer is usually performed in the uterine horn ipsilateral to the corpus luteum.

Transfer of embryos is mostly accomplished by two alternate procedures: either surgical, or non-surgical by laparoscopy (González et al. 1991). In both cases, a puncture is performed on the dorsal side of the uterine horn and in its upper third portion. The embryos are located in the uterine lumen by means of a micropipette (conditioned in 10 ml PBS medium).

There is also a combined technique whereby the uterine horn is visualized using laparoscopy. A small 1 cm incision is made in the abdominal mid-line and the uterine horn is exposed using a clamp, so as

to carry out embryo transfer (semi-surgical embryo transfer) (Photos 9 and 10).

In dairy goats, a higher rate of embryo survival was found after transfer of two embryos per recipient (Moore and Eppleston 1979; Armstrong et al. 1983; Tervit et al. 1983). In sheep, global efficiency (lambs produced/transferred embryos) is greater when one embryo per female recipient is transferred (Cseh and Seregi 1993).

Tolerance in estrus synchronization times between donor and recipient females is ±1 day. When donor-recipient estrus synchronization is optimal, embryo transfer efficiency is increased (Moore and Rowson 1960).

It is important to bear in mind the so called 'donor effect', defined as the variability observed in embryo survival rates (0 to 78%) for embryos of the same quality, from different mothers (Heyman et al. 1987).

Laparoscopic or visual ovary examination of recipients must be performed to ensure that females with one or two corpora lutea corresponding to days 6 or 7 of the estrus cycle are available. In addition, when recipients are selected, it must be remembered that the number of corpora lutea influences embryo survival. Armstrong et al. (1983) reported embryo survival rates of 52, 63 and 75% for recipients with 1, 2 or 3 corpora lutea, respectively. Laparoscopic techniques contribute to a good classification of recipients based on their ovulatory response.

On certain occasions, especially in goats, recipients with cystic follicles or regressed corpora lutea are found. These females must not be used as recipients. Cervical embryo transfer is rarely used owing to difficulties in transposing the cervix (Lewalski et al. 1991; Flores-Foxworth et al. 1992).

It is very important to take into account the time interval between embryo recovery, identification and assessment of embryo quality and the corresponding embryo transfer. Because of the hard work involved in carrying out an ET program, it must be very well organized and coordinated to ensure optimal results.

CHAPTER FIVE

Conclusions

5.Conclusions

Embryo transfer can increase the number of offspring from a genetically superior female, resulting in an average of four offspring per multiple ovulation treatment. Recent advances in the reproductive efficiency of ET have increased the possibility of its being used in genetic improvement programs by furthering the distribution of sheep genes with high productive value. Future research will be required to reduce costs and increase the number of offspring per donor sheep. This will facilitate its commercial application, as has already been accomplished with cattle.

There is no doubt at present time that, for health reasons, ET is the safest method for importing various high production biotypes. The growth of international commerce in genetic material through the use of ET has proved the importance of the technique in providing health guarantees against exotic diseases and, furthermore, as a tool for the improvement of animal production.

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية الطب البيطري

نقل الاجنة في حيوانات المزرعة

(التقنية الحالية لنقل الاجنة في النعاج)

بحث مقدم إلى

مجلس كلية الطب البيطري / جامعة القادسية وهي جزء من متطلبات نيل درجة البكالوريوس في علوم الطب والجراحة البيطرية

من قبل الطالب

أحمد صلاح هادي ملوكي

إشراف الدكتور

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2016

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الخلاصة

SUMMARY

الخلاصة

ان علم الاجنه من العلوم الحديثة التى تستخدم للتحسين الوراثى فى الحيوانات وله دور مهم فى اقتصاديات الانتاج الحيوانى . ويهدف برنامج نقل الاجنه فى الاغنام والماعز الى اجراء التحسين الوراثى والاسراع فيه لانشاء قطعان عالية الانتاج (انتاج توائم – انتاج عال من الحليب) فى فترة زمنية قصيرة وذلك عن طريق نقل اجنة من امهات (نعاج) ذات صفات وراثية ممتازة فى انتاجها (وتسمى نعجه معطية (Donors الى امهـات (نعاج) اخرى متوسطه الانتاج وتسمى نعاج مستقبلية . Recipients ويتم تنفيذ برنامج نقل الاجنه عن طريق عمل ما يسمى بتعدد التبويض Superovulation وهى طريقة تستخدم لزيادة عدد الاجنه المتحصل عليها من النعجه الواحدة (ومن اناث الحيوانات بصفه عامه). وهناك عدة طرق ناجحه لتعدد التبويض . ورغم ان اول عملية ناجحه لنقل الاجنه كانت فى الارانب سنه 1890 الا ان اول عملية لنقل وزرع الاجنه فى الحيوانات المزرعية كانت فى الاغنام 1949 م وكانت اول عملية ناجحة لنقل الاجنه فى الابقار عام 1951 م بالاضافة الى ان اول شركة تجارية تكونت من اجل تنفيذ برنامج نقل الاجنه فى الحيوانات، (الابقار) عام 1971 م .، وللعلم فان اول ولادة لطفلة بعد نقل الاجنه فى الانسان كانت 1978.