

Comparative Study Between Different Methods of Insemination in Iraqi Does

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Summary

Present experimental was carried out on 22 does 2-4 years old. The aim was to induction of estrous in non-breeding season using impregnated sponge with 20 mg of medroxy progesterone acetate (MPA) for 13 days and with an i/m injection of 500 IU Pregnant Mare Serum Gonadotropin (PMSG) hormone 24 hrs before sponge withdrawal and to compare the pregnancy rate using three different methods of insemination; natural , cervical and laparoscopic artificial insemination at fixed time 24-48 hrs after estrous onset. All does were showed signs of estrous (100%), the estrous time was 46.9 ± 4.90 hrs (24-60hrs) after sponge withdrawal. While estrous length was 37.09 ± 1.91 (24–72hrs). All experimental animals followed up by abdominal palpation, ultrasonography at 30, 60 and 90 days post-insemination, while the laparoscopic examination was performed at day 30 post-insemination, to improve the efficiently of the different methods of inseminations in pregnancy rate ultrasonographical diagnosed by rectal and abdominal methods of 14 does post-inseminations appeared that two were pregnant, seven suspected and five non pregnant. At day 60th , post-insemination the pregnancy diagnosis of (22) does appeared that (14) were pregnant, six suspected and two non pregnant. While at day 90, the examination of all does appeared that (16) animals were pregnant and six were not. The laparoscopic examination, at 30 days post-insemination showed that eight does were pregnant, two suspected and two were not pregnant. In conclusion that the pregnancy rate of laparoscopic insemination is (75%), comparisons to natural (66.7%) and cervical AI method (75%) but there is no significant different between them, while the kidding percentage was appear in natural insemination (75%), cervical (100%) and laparoscopic insemination (150%). The results of this study indicate that estrus can be efficiently induced in female goats during non-breeding season using 20mg MPA impregnated sponge with 500IU PMSG.

Keywords: does, cervical, laparoscopic, artificial insemination, PMSG, MPA

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دراسة مقارنة لتقنيات مختلفة للتلقيح في المعز العراقي

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الخلاصة

اجريت هذه التجربة على 22 من المعز تراوحت اعمارها بين 2 – 4 سنة. هدف هذه الدراسة احداث الشبق في اناث المعز خارج موسم التناسل باستعمال الاسفنجات المهبلية المشبعة بـ 20 ملغم من البروجسترون (ميدروكسي بروجسترون استيت) لمدة 13 يوم وفي اليوم 12 قبل سحب الاسفنجة تم حقن 500 وحدة دولية من هرمون مصل الفرس الحامل في العضلة وتم مقارنة طرق مختلفة لتلقيح اناث المعز بالتسفيد الطبيعي , بالقسطرة (عقن الرحم) و بواسطة الجراحة المنظارية بوقت ثابت هو 24 - 48 ساعة بعد ظهور الشبق ولمرة واحدة باستعمال تلقيحة واحدة. اظهرت نتائج توحيد الشبق بان جميع الحيوانات قد اظهرت علامات الشبق واضحة وبنسبة 100% , كان معدل وقت ظهور فترة الشبق من رفع الاسفنجات الى بداية ظهور الشبق (4.9±46.9) بمعدل 24-60 ساعة وطول الشبق 37.09 ± 19.1 بمعدل (24-72) ساعة لجميع الحيوانات تم فحص الحمل بواسطة الموجات فوق الصوتية بعد 30, 60 و 90 يوم من التلقيح بينما تم فحص الحمل بواسطة الجراحة المنظارية بعد 30 يوم من التلقيح. الموجات فوق الصوتية وفي اليوم 30 من التلقيح (اثان حوامل, سبعة مشكوك فيها و خمسة غير حوامل) وبعد 60 يوم من تلقيح, تم فحص الحمل لـ 22 حيوان بواسطة الموجات فوق الصوتية (12 حوامل, ستة مشكوك الحمل و اثنان غير حوامل), بينما بعد 90 يوم من التلقيح, تم فحص الحمل لـ 22 حيوان بواسطة الموجات فوق الصوتية (16 حوامل و 6 غير حوامل) اما نتيجة الفحص بالجراحة المنظارية وبعد 30 يوم من التلقيح فقد اظهرت (ثمانية حوامل, اثنان مشكوك فيها و اثنان غير حوامل) من مجموع 12 حيوان.

نستنتج من هذه الدراسة ان التلقيح بالجراحة المنظارية هو اكثر فعالية من حيث نسبة الحمل والولادات (75%) و (150%) مقارنة مع تسفيد الطبيعي (66.7%) و (75%) والتلقيح بالقسطرة (75%) و (100%) على التوالي كذلك نستنتج امكانية احداث الشبق خارج موسم التناسل باستعمال الاسفنجات المهبلية مشبعة بـ 20 ملغم من (ميدروكسي بروجسترون استيت) وحقن 500 وحدة دولية من هرمون مصل الفرس الحامل.

Introduction

Iraqi goat are multipurpose animal produce milk, meat, skin and hair In mature fertile doe the ovaries are almond in shape and have mulberry – like appearance due to multiple follicles (1). In goat intravaginal sponge contain much synthetic preparation with progesterone activity, and pregnant mare serum gonadotropin (PMSG) could be use for estrous induction and superovulation progesterone and PMSG were used in different doses (2). Artificial insemination (AI) has an important role in goat breeding and to control reproduction (3).

Laparoscopy is a minimally invasive procedure used as diagnostic tool and surgical treatment (4). It is commonly used in gynecology to examine the outside of the uterus, fallopian tubes and ovaries particularly in pelvic region. Ultrasonography consider as easy technique for early pregnancy diagnosis (5). In view of this and since there is little information is available concerning this aspect had been conducted with following objectives: Induction of estrous of Iraqi goat using 20 mg impregnated sponges with Medroxy Progesterone Acetate (MPA) during non breeding season (cold months), and to evaluate pregnancy rate and prolificacy of goats inseminated at fixed time by laparoscope and cervical with fresh diluted semen contain about 100 million active fresh sperm and natural mating, in addition, to determine a best technique of insemination which associated, with minimal damage to health and welfare animal.

Materials and methods

This study was carried out on twenty two healthy does cross breed ranged in age from (2-4) years old and weighted about (35 kg). Induction of estrous in non-breeding season was achieved by insertion of intra vaginal impregnated sponge with 20mg MAP (Medroxy Progesterone acetate) for 13 days synergized with intramuscular injection of 500 (IU) PMSG at 24 hrs before withdrawal a sponge. The animals divided into three groups according to insemination rout: Natural inseminated , cervical inseminated and laparoscopic surgical inseminated group. By using fresh extended semen with TRIS3 (TRIS=hydroxyl methyl amino methane buffer), modified by (6 and 7). The insemination was performed at fixed time 24-48 hrs after estrus onset. Detection of pregnancy at 30,60 and 90 days post insemination based on ultrasonic examination (WELLD®, China) and 30 days post-insemination on laparoscopic examination (Carl Storz Company, Germany). The data were statistical analyzed by SAS program.

Results and Discussion

The does not show any signs of estrous during pre-treatment period which detected by use buck and ultrasonic tests. While during progesterone treatment no signs of estrous were detects. This result agrees with that reported by (8 and 9), which suggested that the insertion of the sponge contained progesterone has the ability to abolish the estrous as long as they exist inside the vagina and considered as artificial source of progesterone insertion. The 20mg MAP used in this work was enough to suppressed the production of gonadotropin of non- breeding season,

the does were showed typical estrous signs within 24-72 hrs after sponge removal (remove of the blockage of progesterone) lead to release of gonadotropin and subsequent estrous and ovulation in female treated with progesterone. In Iraq Kashifalkitaa (2) recorded 100% of estrous emergence after using 40mg MAP impregnated sponge in goat. While, (10) used 40 mg progesterone in does during breeding season and recorded 77.55% estrous synchronization and 60 mg during non breeding season recorded 33% emergence estrous. This variation could be due to the high level of progesterone 60 mg and long treatment, which led to decrease the fertility of animals, which effects on hypothalamus – ovary axis. Present result of induction of estrous (Table 1), it is initial that the time of estrous emergence after sponge withdrawal was 46.9 ± 4.90 hours, while the mean of estrous length was 37.09 ± 1.91 hrs, this indicated that the correct dose is 20mg of MPA accompanied by 500 IU PMSG used in non–breeding season. Like a present work, Smith, (11) describe that the ovulation in the female goat usually occur 30-36hrs after onset of estrous. Also, the same result were described when the PMSG hormone given 24 hrs prior to the sponge removal during non breeding season and the does come to heat within 12-36 hrs after sponge displacement. And the insemination will be take place at 48 hrs of estrous onset (12 and 11). These results were summarized in Table 1.

Table 1: The time of estrous emergence after vaginal sponges removal and the duration of estrus (Mean \pm SE / Range-hrs)

Group	No of animals	The time of the estrus emergence after sponges withdrawal (hours)	The length of estrus (hours)
A	6	48 ± 4.39 / 36-60 _a	38 ± 37 / 24-48 _b
B	8	48 ± 4.54 / 24-60 _a	36 ± 35.4 / 24-48 _b
C	8	45 ± 4.40 / 24-60 _a	37.5 ± 35.4 / 24-48 _b
Total	22	46.90 ± 4.90 (24-60)	37.09 ± 19.1 (24-48)
LSD		1279	103

Different small letters represented significant different at the level of (P<005) in between group A, B and C

The present protocol was also recommended outside of breeding season to improve ovulation rate by (13 and 14). Armstrong (15) and Drion (16) are claimed that PMSG has a long half life about 4-6 days in turn, the long action of biological activity causing to continually release of a few number of non ovulated immature follicles. The sponge complications occurred in two treated does during sponge withdrawal, part of sponge adhered in vagina wall and by along artery forceps the remainder part of sponge was pullout without any effect on the fertilization and pregnancy. Similar observation was reported by (17 and 18) that found the adhesion of sponge to the vaginal wall will cause profiles vaginitis and odema, the

adhesion maybe due to narrowing, short vagina and long period of sponge insertion.

In this study, the insemination dose contained about 100×10^6 active sperm deposited in uterus through both laparoscopic and cervical, which give good results. Table 2 Since the low twining percentage could be due to onetime insemination used by the present work while pregnancy rate which was reported by (19) found a pregnancy rate is 59.4% after cervical artificial insemination, using fresh semen insemination in dose of 100×10^6 spermatozoa, as well as the inseminations occurred at fixed time between 24-48 hours after withdrawal of progesterone sponges. The differences of pregnancy rates depending on the method of insemination and the insemination dose and co-treatment as well as to avoid animals stress and embryo damage (20).

There were no differences between the pregnancy rate recorded in does were insemination by laparoscopic and cervical routes (Table 2). This result is consistent by (21). The cervical results are different with that reported by (2) in Iraq who found the proportion of natural and artificial insemination 60 % and 61.54 % respectively.

While laparoscopically results are consistent with (22) in Iraq sheep 71.4%. And these results were differed with (21 and 23), which were 53% and 44.89 % respectively.

Present results of pregnancy by different method could be indicated that the cervical insemination used cause neither injury to the vaginal mucosa, nor discomfort when does were subjected to vagina speculum and plastic catheter, there was no complication and all the semen that was deposited in cervix was in place Laparoscopically deposition of semen directly to the uterus with less postoperative infection, pain and softy for reproductive organs.

Table 2: Pregnancy percentage at 90 days post insemination using ultrasound.

Type of inseminations	No of animals	Pregnant	Percentage	Kidding %	Non pregnant	Percentage
A	6	4	66.7% _a	75% _a	2	33.33%
B	8	6	75% _a	100% _b	2	25%
C	8	6	75% _a	150% _c	2	25%
Total	22	16	72.72%	32.5%	6	27.27%

$\chi^2 = 15$, different small letter represented significant different at the level of (P<005) in between group A,B and C

Table 3: Pregnancy diagnoses obtained from the ultrasound and laparoscopic at 30,60 and 90 days post insemination (N=22)

Diagnosis	Post insemination day 30		Post insemination day 60	Post insemination day 90
	Ultrasound / 14 does (35 MHz)	laparoscopic surgery / 12 does	Ultrasound / 22 does (35 MHz)	Ultrasound / 22 does (35 MHz)
Positive	2	8	14	16
Suspected	7	2	6	Zero
Negative	5	2	2	6
Total	14	12	22	22

The results of the pregnancy diagnosis showed that 14 does at day 30th (Table 3) using ultrasonic, 2 pregnant, 7 suspected pregnancies and 5 non pregnant does this result indicated insufficient of 35 MHz probe for early pregnancy diagnosis. Laparoscopic examination was used to diagnosis of pregnancy in 12 does, 4 of them were laparoscopic inseminated, the result revealed 8 pregnant and 2 does suspected and 2 non pregnant does (Table 3). The laparoscopic early pregnancy diagnosis revealed the importance of this method for pregnancy diagnosis in small ruminant, after 60 days of insemination, all animals were examined using ultrasonic method (through abdominal wall examination only), the results were explain in Table 2. This result indicated that 35MHz probe is sufficient for pregnancy diagnosis at day 90 of insemination, the results were: 16 pregnant and 6 non pregnant (Table 3).

The results of pregnancy rate Table 3 Indicated that the using of fresh diluted semen is successful in cervical AI as well as in the laparoscopic surgical technique, semen placed directly in the uterine body The insemination directly to the uterus is gave a good result as compared to the vaginal insemination (23) reported that the high percent of pregnancy by surgical laparoscopic and claimed the laparoscopic technique is difficult, with relatively high cost and repeat insemination also interfere with the animal welfare. Present work could agreed with (24) suggestion but the laparoscope method could be interfere with the animal welfare. During this work four of does were laparoscopic inseminated were checked by laparoscopic for pregnancy diagnosis and had offspring with normal gestation duration, while, Anel (23), using cervical insemination with vaginal dilators in does reported uncomfortable position at the time of insemination, recorded 10% lower pregnancy rate. In this study the duration of gestation period was 151.58 ± 28.0 days. This result is in agreed with (25). Out of 16 does only 4 does carrying twin (25%). The pregnancy rate was 72.72% (16 out of 22 does). The low twin percentage could be due to the high dose of PMSG used in present work in non-breeding season which lead to increase in the ovulation of immature follicles, with increase of estrogen hormone that induce increases in serous vaginal secretions and discarding of sperm outside the vagina. As well as once insemination with 100 million sperms could be not enough to produce twin pregnancy in does when inseminated in out of breeding season.



Figure – 1:Laparoscopic insemination, insertion of needle in the doe uterus.



Figure – 2: Laparoscopic examination at 30th day post insemination.

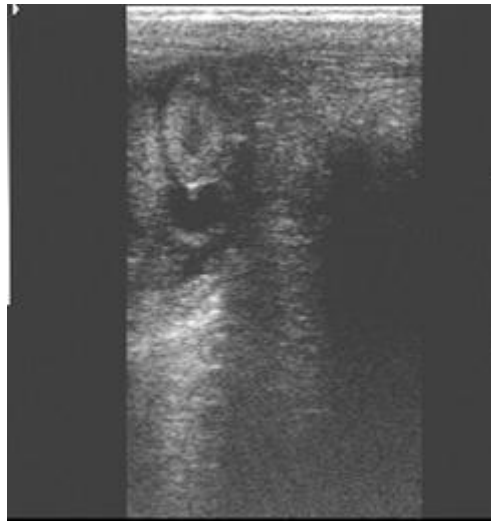


Figure – 3: Ultrasonic diagnosis of pregnancy at 30th day post insemination.

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