Non-Surgical Collection of Embryos from Iraqi Local Goats

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Abstract

Seven does were used to study the non-surgical method of embryos collection, superovulation was induced by using PMSG (450 I.U) the day of natural mating is considered-day zero. The dose were bred from fertile bucks at the appearance of the oestrous signs, the day of natural mating is considered-day zero, the embryos were collected at the sixth or seventh days after the day zero. The process of embryo collection was utilized in standing position after epidural anaesthesia. Results of the study revealed that one embryo in morula stage (33%) were successfully collected in day 6 post mating and three embryos (75%) were recovered on day 7 post mating.

جمع الأجنة بالطريقة الغير الجراحية ونقلها في المعز العراقي

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الخلاصة

استخدمت في هذه الدراسة 7 معزات. تم جمع الأجنة منها باستخدام الطريقة الغير الجراحية بعد ان تم استحداث فرط الإباضة باستخدام برنامج هرمون المحفز نمو الجريبات (450 وحدة دولية) (المترودين) واعتبر يوم التلقيح هو يوم الصفر. لقحت المعزات من ذكور المعز خصبة عند ظهور علامات الشبق عليها. جمعت الأجنة في اليوم السادس أو السابع بعد التلقيح الطبيعي من قبل الذكور. حيث تم جمع الأجنة والمعز في وضع الوقوف بعد تخديرها فوق الجافية. تم الحصول على جنين واحد في مرحلة التوثية في اليوم السادس بعد التلقيح (33%). وثلاثة أجنة (75%) تم الحصول عليها في اليوم السابع بعد التلقيح.

Introduction

Embryo recovery in goat has been utilized using a surgical (1,2), non surgical (3) and Laparoscopic technique (4). Disadvntage of the surgical approach are the stress to animal and adhesion forming that limit the number of times it can be flushed the same animal and lower the future fertility of the animal (5,6). Laparoscopic embryo collection results in fewer adhesions, but requires special equipment, and an experts personal (4,5). Meanwhile, there has been limited success utilizing non-surgical methods of embryo recovery in goats (7). Non-surgical embryo collection has always been hampered by difficulty of introducing a catheter through the cervix and the in capability of rectal manipulation of the reproductive tract (8). For these reasons, several types of catheters have been used with this approach. The internal cannula of verris needle has

been described by Amoah and Gelaye (6) for passage through tight cervices while Kraemer (9) mentioned the used a 14-ga angio catheter with blunted stylet. Goel, et al. (10) used a two ways 24 G Foley catheter through which a stainless tube is inserted. Foly catheters utelize a balloon to seal the internal cervical. There is no available reports describe application of the simple catheter (Without inflatable cuff) which are used in the present study. The current study has been performed to used the non-surgical method (with modification) to recover embryos in local Iraqi goats.

Materials and Methods

A total number of seven goats of local Iraqi breed aged 3-4 years used as embryo donors in year 2001. All goat, were subjected to a superovulatory regimen using PMSG regimen (Fig. 1). Superovulatory treatment consisted of 1000 I.U of PMSG (intervet international B.V. Boxmeer- Holland) injected I.M on the day of removal of progesterone intravaginal sponges (Sanofi Saute nutrition animal-La Ballastere-France). A single injection of 100 I.U of hCG (intervet international B.V. Boxmeer-Holland) was given at the time of estrous signs. The goats were mated (Hand mating) with fertile male twice daily as long as they exhibited standing oestrous. Non-surgical embryos recovery were performed on either day 6 or day 7 after the last mating. The equipment which used for non- surgical embryos collection consisted of suitable vaginal speculum two Allis forceps pocket flash light, flushing catheter without inflatable cuff provided with stainless rod, 50 ml disposable syringe, large petri dishes (94x16mm) (Fig.2). Low epidural anaesthesia was performed on all donor goats using of 2% Lidocaine. Uterine flushing was utilized and the goats was in standing position. Goat were restrained in standing position with two persons the lubricated speculum were inserted into the vagina after the genital area were carefully cleaned-the lip of the external os of the cervix was grasped with the Allis forceps (Fig.3) The speculum was removed and the external os was carefully pulled caudally until if almost reached the vulvar opening. The flushing catheter with its stainless rate were introduced into the cervical canal by gentle probing and twisting motions (Fig.4). The tip of the catheter was directed either into the left or right uterine horns with a finger in the vaginal fornix. Once the catheter was introduce into the desired uterine horn, the stainless rode was pulled and the free end was connected to disposable syringe filled with 20 ml of phosphate buffer solution PBS. A total volume 90-100ml of flushing solution was infused into the uterus of each goat using 20 ml disposable syringes Fig (5). Meanwhile the flushing solution was collected immediately from the uterus using the same syringes. The collecting flushing solution. A total of 5 flashings was performed for each goat. The recovered fluid was emplied into large petri-dishes that were covered and kept at 30°c in incubator. The embryo were identified using sterio microscope at x 20.

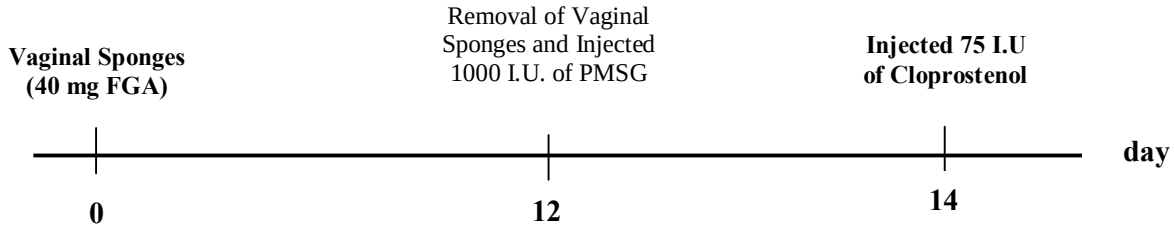

Fig. (1) Superovulatory of non- surgical flushing regimen

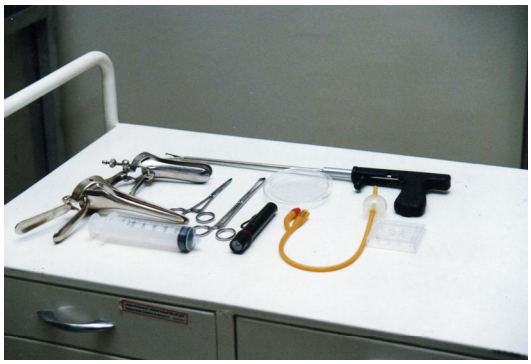

Fig. (2) Tools used for non-surgical embryo collection

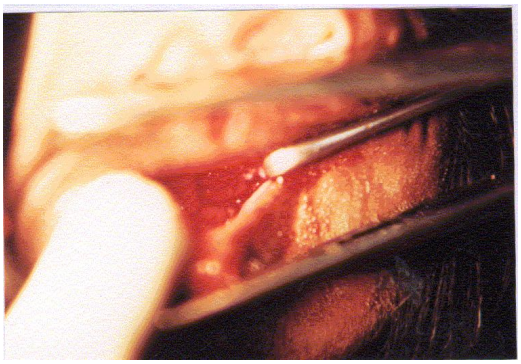

Fig. (3) Allis forcips grasping the external os of cervix

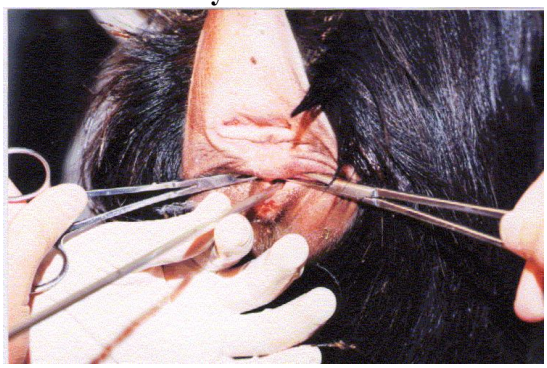

Fig. (4) Flushing catheter introduce through the cervical canal

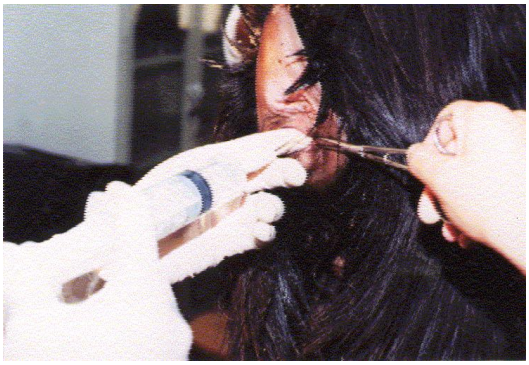

Fig. (5) Infuse the flushing solution inside the uterus through flushing catheter

Results

A total number of 5 embryos were collected in the present study. These embryos were collected from goats 1, 2, 6 (one embryo from each) and goat 6 (two embryos), (table 1). The percentage of recovered flushing solution from these goats (1, 2, 5, 6) were 75, 75, 90 and 80% respectively (Table 1). Where as no embryo were collected from goats 3, 4 and 7. The percentage of recovery rates flushing solution from these goat were 50, 37.5 and 27.7% respectively (Table 1). The result showed that all recovered embryos which are collected on day 6 and 7 post mating were at morula stage (Fig 6). The results revealed that high percentage (75%) of embryos were collected at 7day post mating (Table 2).

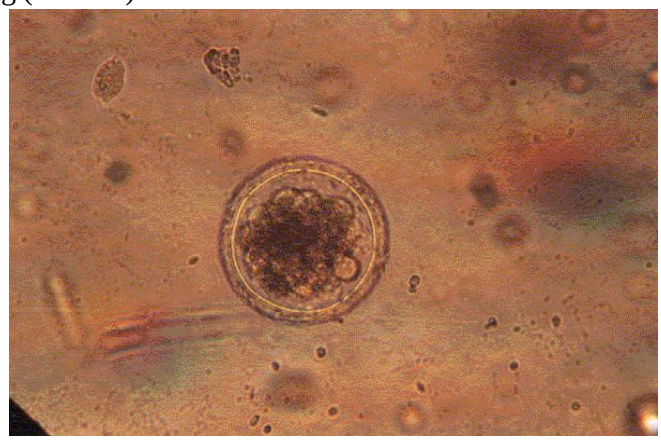

Fig. (6) Collected embryo in Morula stage (16-32 cell) (10x)

Table (1) Relation between percentage recovered flushing fluid and number and development stage of recovered embryos

Goat	1	2	3	4	5	6	7
Day of collection	7	7	6	7	7	6	6
Volume of Flushing fluid	80	100	100	80	100	100	90
Volume of recovered flushing fluid (ml)	60	75	50	30	90	80	25
Recovered fluid (%)	75	75	50	37.5	90	80	27.7
Number of recovered embryos	1	1	-	-	2	1	-
Development stage of recovered embryos	Morula	Morula	-	-	Morula	Morula	-

Table (2) Relationship between the day of flushing and the percentage of goats successfully embryos recovery

Day of collection	Number of goats subjected for flushing	Number of goat with successfully embryo recovery	%
6	3	1	33
7	4	3	75

Discussion

Results of the present study showed that five embryos are recovered successfully by using the present non-surgical procedure. Although few number of embryos had been collected, this results indicated the capability of complication and stress to the animal. Meanwhile the number of recovered embryos were less than number of recovered by other studies using surgical and laposcopical techniques (11,12). Similar results were recorded by (10) using the non-surgical method. The fewer number of embryos recovered in the present study could be due to the type of the flushing catheter which was not provided with inflatable cuff to prevent the flushing fluid from tranfer to other horn and cervix (6). The results indicated that the recovered embryos were collected from uteri of goats from which high percentage (75-98%) of flushing solution was recovered. This result agree with Goel, et al. (10). In this regard, good attention should be given to collect large volume of flushing solution in order to collect good number of embryos. The results indicated that good number of embryos have been collected on day 7 postmating. Similar result has been recorded by Pereira, et al. (8).

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