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Sex Determination of in Vitro Produced Goat Embryos by Epididymal Spermatozoa Selected by Swim up Technique

Sura Shakir Hammood

Department of Surgery and Obstetrics, College of Veterinary Medicine/Baghdad University

Abstract

The study was conducted to determine the sex of the male and female goat embryos produced in vitro fertilization by Epididymal spermatozoa of local buck and separated by swim-up technique. The sex of produced embryo was identified by polymerase chain reaction (PCR). Results indicate that the fertilization by Epididymal spermatozoa selected using Swim-up technique at 200×g centrifugation showed that 48.70 ±0.62 % of spermatozoa were detected in the supernatant while the precipitate contained 42.1±0.70. Spermatozoa in the supernatant were used for IVF of matured oocytes. The sex of goat embryos produced in vitro fertilization was determined by polymerase chain reaction (PCR) using specific primers to detect the SRY gene. The percentage of male embryos recorded 71.73% while female embryos recorded only 28.26% from the total goat embryos obtained after IVF by sperms selected using swim-up at centrifugation force of 200×g. It was concluded that the use of swim-up technique up at centrifugation force of 200×g on Epididymal spermatozoa showed the ability of selection male embryos in caprine.

Key words: *Swim up, Gender, Goat, Cauda, Epididymis, Embryo, SRY Gene.*

Introduction

The animal manufacture depends upon increase in the number of productive livestock and a major concern is to increase the efficiency of offspring from them. The goat plays a significant role in socioeconomic development because of its contribution of milk, meat, skin and fur to humans and plant nutrition as farmyard manure, being small in size and having a short gestation period [1]. Predetermining the sex of animals has been a central goal of producers for generations because of its economic advantage. The current development of state of the Assistant Reproductive Techniques (ART) has made it possible to predetermine the sex, involving the separation of X- from Y- chromosomes bearing sperms, used in artificial insemination (AI), in vitro fertilization, and embryo transfer [2]. Sperm sexing has progressed from research to commercial application for humans and cattle [3.] Sperm separation methods are capable to significantly improving sperm quality with a high rate of progressive motility and morphological normal spermatozoa [4]. Several investigators have attempted to separate X- and Y spermatozoa using diverse techniques based on principles of differing mass and motility, swimming method, surface changes, centrifugal countercurrent distribution, immunologically relevant resources and volumetric differences [(2,5)]. The potency of sex preselection in semen has been demonstrated for multiple species, including endangered species in zoos and aquarium animals [6]. The objective of this research is the separation of X- from Y-bearing Epididymal spermatozoa of local goat by swim-up technique which with can be simply appropriated in small laboratory and with least laboratory appliances.

Materials and Method

Gonads of local goat were collected from Al-shu'alah abattoir and transported in a normal saline by cool box within 30 minutes to the laboratory. The ovaries were removed and washing three times with fresh normal saline and twice with a collecting media (TCM-199, TALP and MEM) and gentamycin to get out of contamination and were sliced into small pieces in fresh normal saline with a surgical blade. The oocytes have been collected in the Hood cabinet by aspiration, slicing or puncture from the normal saline solution to MEM according to procedures of Wani et al10. Aspiration of 2-8 mm size follicles were aspirated with 18 gauge needle attached with a sterile 3 ml disposable syringe containing 2 ml of collecting medium and the

slicing was performed by placing the ovaries in a sterile Petri dish containing 10 ml of collecting medium, held with forceps and the ovarian surface were incised with a scalpel blade while puncture visible follicles on the surface of the ovaries ranging from 2-6 mm in diameter with 18- gauge syringe needle. [(7,8,9]) The media with harvested oocytes were transferred to one petri dish [(10 ,11).] The wells of the dish were examined under inverted microscope and then the total number of oocytes was counted. Collected oocytes were examined and graded according to [12] as grade A, grade B and grade C on the foundation of cumulus cells and uniformity of cytoplasm.

Only grade A, and grade B oocytes were selected under a stereomicroscope, washed twice with cultured medium, and incubated in appropriate maturation medium at 39 °C, 5% CO_2 and 90% relative humidity for 24 hrs, the numbers of matured oocytes were calculated. Every 5 oocytes were placed in a well out of 24 wells dish containing 2 ml of the medium MEM containing HEPES buffer, sodium Bicarbonate, crystalline penicillin, streptomycin, fetal calf serum 10%, was used as maturation medium, presence of first polar body was the criteria of oocytes maturation. [13.]

Sperm separation by Swim-up technique:

The procedure of the modified swim-up technique is essentially the same as that described by [12], Ham's F10 (Euro-Lone, Italy) was used as an substitute media to estimate the effectiveness of the swim-up technique in separating X and Y chromosome bearing sperms. Sperms sample transferred to 10 ml centrifuge tube. Sperms washed twice using 0.5 ml Ham's F-10 medium then centrifuged at 300×g or 200×g for 10 minutes. Supernatant removed and overly sperm pellets with 0.5ml of Ham's F-10 medium in each tube. The tubes were put in the incubator, inclined at an angle around 45° and incubated at 37°C and 5% CO_2 for 60 minutes. By inclining the tubes at 45 to ameliorate the capability of the sperms to swim out of the sample and reaching the medium. Following incubation, the first 0.25ml was discarded and the final 0.25ml was used for IVF after sperm evaluation [12].

Groups of matured oocytes were kept in of 5 in petri dish containing media MEM supplemented with 10% heat inactivated estrus goat serum (EGS), BSA and antibiotics (Penicillin and Streptomycin) for fertilization with sperms and incubated at 39 C, 5% CO_2 and 90% relative humidity for 24-27 hrs [13]. After fertilization at 24-27 hrs, oocytes have 2nd polar body or oocyte with head of sperm in the cytoplasm was evaluated as fertilized oocyte. [13].

Cultures of fertilized oocytes were performed; embryos were cultured in MEM at 38.5-39 °C, 5% CO_2, and 90% humidity. Every 24 hrs, developments of embryos were perceived, 50% of the media volume was replaced with fresh medium at 24 hrs intervals according to [14] procedure, and then DNA extraction from embryos was applied.

DNA extraction from cultured embryos:

The primers (Promega, Germany) were used to detection SRY gene, they prepared according to the details of the producer. The sequences and product size of the primers are:

The forward primer sequence was: ATGAATAGAACGGTGCAATCG

(OD-260: 12.9, Microgram: 382, Pico moles: 58704)

The reverse primer sequence was: GAAGAGGTTTTCCCAAAGGC

(OD-260: 11.7, Microgram: 363, Pico moles: 58842)

The Statistical Analysis System- SAS (2012) program was used to influence of difference factors in parameters of study. Chi-square test was used to significant comparison between percentage and least significant difference –LSD test (ANOVA) was used to significant comparison between means in this study.

Results and Discussion

Several techniques were used for the collection of oocytes from the ovaries in goats. The effect of collection methods on the recovery rate of oocyte is shown in table (1). Aspiration technique showed oocytes recovery rate of 62% (81/131) with a mean of oocytes per ovary and Perforation (puncture) showed oocytes recovery rate of 57% (71/125) while slicing showed oocytes recovery of 48% (55/114). There was a significant difference ($p<0.05$) between different collecting techniques. Recovered ova via aspiration and puncture are statistically different than ova recovered by slicing. Similar observation was shown by [15] while [16] and [17] in the sheep. The results disagreed with [9] in goat and [12] in sheep. The aspiration

and puncture methods recorded a high recovery rate due to the aspiration and puncture considered as the applicable technique for obtaining perfect oocytes quality and quantity production , while the presence of the ovarian tissue debris in the slicing due to destruction the ova during the examination [13].

Table (1): Effect of collection methods on recovery rate of ova

Methods	Ovaries numbers	Ova numbers	No. of Recovered oocytes	Recovery rate percent
Aspiration	50	131	81	62%a
Slicing	50	114	55	48%b
Perforation(puncture)	50	125	71	57%a

Different superscripts showed significant difference (P<0.05).

Table (2) showed the type of collection methods on the grade of the ova recovered. Results showed that higher recovery rates were obtained from grade B in all collection methods as compared with grade (A) or grade (C). Corresponding observation was shown by [15] in goats. Wani et al [12] have made comparable observation in sheep and [18] in goats. The low quality grade oocyte recovered could be due to slaughters of low-quality does.There were statistically significant differences (P <0.05) in oocytes quality between aspiration and puncture as compared to the slicing method.

Table-2-: Effect of collection methods on the grade of oocytes (oocyte quality).

Type of collection method	**No. of ovaries**	**Grade (A) %**	**grade (B) %**	**Grade (C) %**
Aspiration	50	40a	65a	26b
Slicing	50	29b	51a	34b
Puncture	50	42a	61a	22b

Different letters reveal a statistical variation (P<0.05).

Table (3) at centrifugation 200 ×g there was significantly higher (P <0.05) in sperm number after washing, sperm number at the top of the tube and sperm number lost than in centerfigation300 ×g, while the number sperms in the bottom part of centrifugation 300 ×g were significantly higher than centrifugation 200 ×g (P<0.05). The mean of sperms lost through the centrifugation procedure at 200×g and 300 × g was 6.06 ± 0.41 % and 3.18±0.86 % respectively and there was a significant difference between two groups (P<0.05).

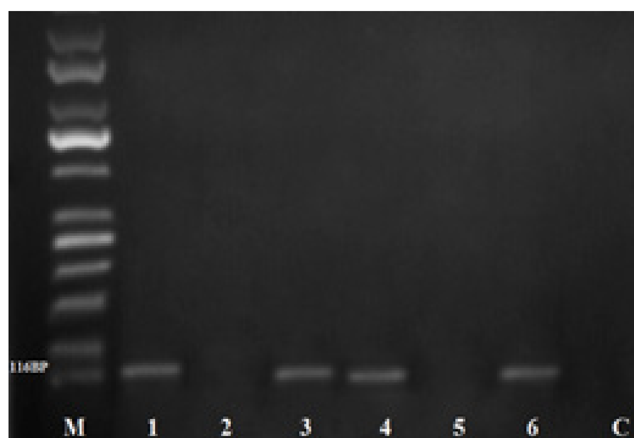
Table 3: Number of sperms used in swim-up technique after centrifugation at 200 × g or 300 ×g

centrifugation	After washing 2 times % of sperms	% of sperms in the upper part of the tube	% of sperms in the lower part of the tube	% of sperms lost
(at 200 ×g)	90.8±0.38 a	48.70 ±0.62a	42.1±0.70b	6.06 ± 0.41 a
(at 300× g)	87.82 ±0.55b	42.32±0.51b	45.50±0.91a	3. 18±0.86b

Table 4: Maturation and fertilization rate of grade A and B oocytes using sperms obtained by swim-up technique in goat:

Cultured oocytes			Total maturation rate No and %	Total Fertilized oocytes No and %
Total oocyte no	Grade A No and %	Grade B No and %		
243	142 (58.43%)	101 (41.56%)	125/243 (51.44 %)	78/125 (62.4%)

In this research, for amplification genomic DNA of in vitro produced embryos of local goat, we were used the primers for SRY gene of caprine, and the PCR products were separated on 2% agarose gels. The result showed that amplification fragment of size 116 bp, the embryos were successfully amplified and when one band dyed by red gel the embryos recorded as male embryos while the result which showed the nonappearance of such bands recorded for the produced embryos as female goat embryos (Figure 2).



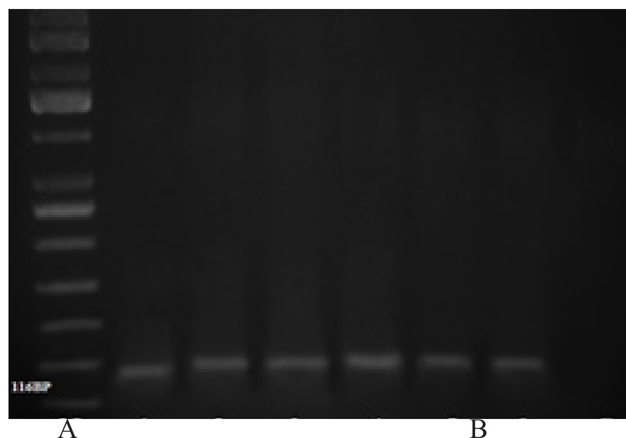


Figure 2: PCR band pattern of SRY gene with 116 bp size of in vitro produced goat embryos, Amplified DNA stained by red gel in the 2% agarose gel. PCR amplification product in: A- columns 1, 3, 4, and 6 indicated the male embryos while the in column 2 and 5 indicated the female embryo. In B- all column indicated the male embryo.

The caudal spermatozoa selected from the upper layer of swim up technique, gave 71.73% male embryos and 28.26% female embryos for determination of the sex of IVF produced local goat embryos. Because X sperm has more DNA than Y sperm, the Y chromosome

is faster than the X chromosome which results in different movement rate [29]. Some authors described that supernatant of swim-up procedure contained more Y sperms [(30,31)]. In this study we used accurate protocol for embryos sexing by PCR, it based on detection of the presence of SRY gene. The Y-specific target sequence amplification only was adequate to identify the gender as has been done effectively in bovine and caprine embryos sexing studies [(31-32).]

Table (4) shows the percentage of male and female goat embryo after IVF by spermatozoa and sexing by using PCR the result disclose that 33 (71.73%) from 46 sexed caprine embryos were male, while female embryos revealed only 13 (28.26%) with a significant difference between the two detected sex (P<0.01).

Table 4: Number and percentage of male and female local goat embryos after obtained by IVF using caudal sperms selected by swim-up technique and detection of sex by PCR.

Technique	No of embryos	No of sexed embryos	No and % of Male embryos	No and % of Female embryos	Chi-Square
Swim-up (200×g)	78	46	33 (71.73%)	13 (28.26%)	12.644 **
** (P<0.01).					

After used swim up technique, the percentage of male embryos evaluated high compared with the results of Marco-Jiménez and Vicente [33] using swim up technique in ovine, and that of Shnawa, (2013) who obtained 81.80 % of male embryos after swim up technique in ovine.

Conclusion

From these data it can conclude that it is feasible to reaping Y bearing spermatozoa from epididymis cauda of local buck by swim up technique (200×g) and use these sperms for IVF to get a high percentage of male embryos successfully.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine/ Baghdad University and all experiments were carried out in accordance with approved guidelines.

References

1. Zarazaga LA, Guzman JL, Domminguez C. Effect of plane of nutrition on seasonality of reproduction in Spanish Payoya goats. Anim Reprod Sci. 2004;87:253-267.
2. Prasad S,Rangasamy S, Satheshkumar S. Sex preselection in domestic animals Current status and future prospects. Veterinary World, 2010;3: 346–348.
3. Rath D, Johnson LA. Application and commercialization of flow cytometrically sex – sorted semen. Reprod. Dom. Anim. 2008;43(2): 338 – 346.
4. Garcia JF. Practical considerations of embryo manipulation: preimplantation genetic typing. Theriogenology, 2001;56:1393-1399.
5. Jain A, Yathish H M,Jain T, Sharma A. Efficient production of sexed semen by Flow Cytometry: A Review, Agricultural Review, 2011;32: 36–45.
6. **Seidel Jr GE. Sexing mammalian sperm-intertwining of commerce technology, and biology. Animal Reproduction Science, Amsterdam, 2003;79 (3-4): 145-156.**
7. Palamo Palamo M J, Izquierdo D, Mogas T, Paramio MT. Effect of semen preparation on IVF of prepubertal goat oocytes. Theriogenology, 1999;51: 927- 940.
8. Al-Timimi IH.Using of Epididymal Sperms of Local Iraqi Bucks for In Vitro Fertilization. Al-Qadisiyah Sci. J. Vet. Med. 2013;12(2):126-132.

9. Saleh WM. Role of Epididymal Spermatozoa In Vitro Fertilization and Embryo Transfer in Iraqi Sheep. PhD thesis in Theriogenology, College of Veterinary Medicine/ University of Baghdad. 2016.
10. Saleh WM. Role of Epididymal Spermatozoa In Vitro Fertilization and Embryo Transfer in Iraqi Sheep. PhD thesis in Theriogenology, College of Veterinary Medicine/ University of Baghdad. 2016.
11. Wang ZG, Xu ZR , Yu SD. Effect of oocyte collection techniques and maturation media on in vitro maturation and subsequent embryo development in Boer goat. Czech Journal of Animal Science, 2007;52: 21-25
12. Wani NA, Wani GM, Khan MZ, Salahudin S. Effect of oocyte harvesting techniques on in vitro maturation and in vitro fertilization in sheep. Small Rumin. Res. 2000; 36: 63-67.
13. Kharche SD, Goel P ,Jha BK, Goel AK, Jindal SK. Factors influencing in-vitro embryo production efficiency of caprine oocytes: A review Central Institute for Research on Goats, Indian Journal of Animal Sciences . 2011;81 (4): 344-61.
14. Keskintepe L, Simplicio AA, Brackett B G. Caprine blastocyst development after in vitro fertilization with spermatozoa frozen indifferent extenders. Theriogenology; 1998;49:1265-1274.
15. Majeed AF. In vitro maturation and fertilization of black local goat oocytes: A comparative study. PhD thesis in Theriogenology, College of Veterinary Medicine/ University of Baghdad. 2012.
16. Sogorescu E, Zamfierescu S, Anghel AH, Dorina N. The influence of new media on the developmental competence of goat and sheep oocytes.Romanian Biotech. Letters. 2010;15 (3): 19- 25.
17. Majeed AF, Omar AA, Alrawi STJ, Al-Maadhidy Assif H, Ahmed KD, Mohamed K. Effect of collection methods on oocyte recovery rate. Research Journal of Biotechnology, 2019;14.
18. Rahman ANMA, Bin Abdullah R, Wan-Khadijah WE. Effects of oocyte source on the developmental competence of in vitro matured goat oocytes fertilized by the intracytoplasmic sperm injection technique, Turkish Journal of Veterinary and Animal Sciences, 2009;33(4):323–331.
19. Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Bioch. Biophys. Acta., 2000;1469:197235.
20. Anton E, Krawetz SA. Spermatozoa as biomarkers for the assessment of human male infertility and genotoxicity. Syst Biol Reprod Med. 2012;58:4150.
21. Wolf CA, Brass KE.The effect of sperm selection by Percoll or swim-up on the sex ratio of in vitro produced bovine embryos. Anim. Reprod., 2008;3(4): 110-115.
22. Lucio AC, MV Resende MV. Assessment of swim-up and discontinuous density gradient in sperm sex pre selection for bovine embryo production. Arq. Bras. Med. Vet. Zootec, 2012 ; 64 (3):525-532.
23. Inaudi P, Petrilli S, Joghtapour A. Reduction of steps in the preparation of motile sperm for intrauterine insemination does not reduce efficacy of the procedure: simplified one-step swim-up method versus classic swim-up. Hum. Reprod. 2002;17: 1288-91.
24. Jamil H, Samad HA, Rehman NU. In vitro Maturation and Fertilization of Riverine Buffalo Follicular Oocytes in Media Supplemented with Oestrus Buffalo Serum and Hormones. Acta Vet. Brno. 2007;76:399-404.
25. Saha S, Khandoker M. Effect of Fresh and Frozen Semen on in vitro Fertilization and Subsequent Development of Goat Embryos. Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh. 2014.
26. Ahmed S, Jalil SJ ,Rahman Z. Impact of gathering procedures on cumulus oocyte edifices (COCs) recovery, In Vitro development and fertilization of goat oocytes. Int. J. Ani. Breed. and Genet., 2015;2 (3):87-90.
27. Shnawa SH. Gender Selection in Iraqi Sheep. MSc Thesis in Theriogenology, College of Veterinary Medicine/ University of Baghdad. 2013.
28. Zeinoaldini S , Jafari Z , Sarmast F , Torbati E , Dadashpour Davachi N. Different harvesting techniques used in ovine in vitro embryo production. 2013.