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Evaluation of different preservative media upon integrity and activity of abattoir ram caudal spermatozoa

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Abstract
The present study is designed to evaluate different preservative media that used in the collection of caudal spermatozoa from abattoir specimens. Seventy abattoir testicles samples are collected from Al-Shoáalla slaughterhouse North-West of Baghdad during the period from November 2015 to April 2016. The total number is divided into seven aliquots (ten samples each). For each aliquot one of the seven media (N. saline, MEM, TCM-199, Glucose, MEM+ N. saline, TCM-199+ N. saline and Glucose+ N. saline) is used to estimate the dead, alive and motile spermatozoa. Time between slaughtering and processing in the lab and effect of transport temperature also studied. In conclusion the differences were not significant among the different media in which normal saline is the cheapest and more practical one. The period among samples collection and lab processing must be limited to minimal period. The more suitable temperature upon specimens transport is between 4-8°C.

Keywords: Epididymis, testicule, spermatozoa

1. Introduction
The need to preserve and utilize epididymal spermatozoa in the most efficient manner is of the utmost importance. Spermatozoa through their release from the 1st of the three parts divisions of the epididymis, the Proximal (Caput), are not motile when diluted with saline, but when the release from the distal (Cauda) are fully motile and gain the ability of fertilization, for this event a great focus has been attributed upon the preservation of the cauda epididymal spermatozoa and the importance of these cells as a tool to conserve Biodiversity [1]. Interest in using epididymal sperm from domestic species is increased, because of the inherent value of individuals who die before making a contribution to the genetics of their own population. However, due to unpredictability of death and often the remoteness of valuable / exotic males, or animals kept in captive condition at the time of death, harvesting and cryopreservation of epididymal sperm in a timely manner are not often feasible [2]. Hence, to maintain the quality of slaughtered / postmortem collected epididymal sperm, preservation methods need to be developed through Artificial Reproductive Technologies (ART) to replenish the gene pool of wild and captive animals that can further increase the utility of sperm [3] Postmortem specimens have a finite time period before decomposition affects functionality, determination of this window of opportunity to harvest and preserve epididymal sperm would be beneficial for further research in sperm preservation and assisted reproductive technologies [4]. In live animals, cauda epididymidis provide a suitable environment for the immature spermatozoa to become mature that acquire motility, spermatozoa recovered from cauda epididymis become motile only when contact with seminal fluid or media [5], and remain functional even hours after the death of an animal [6]. The role of these Epididymal secretion in maintaining its vitality and to permit the development of spermatozoa motility and possibly to protect them from noxious agent [7]. This is mean that; the function of different media which are used to preserved, protect and maintained spermatozoa activity and motility are just a kind of diluents to the sections of the different epididymal cells. Epididymal duct is now recognized as a channel that transports, concentrates and stores the spermatozoa [8]. It is well known that; spermatozoa when leave out the testis are immotile, immature and unable to fertilize an oocyte [9], and that under androgen control, the epididymal epithelium cells secreted proteins within the intraluminal compartment that create a very complex environment surrounding the spermatozoa [10, 11].

Several types of epithelial cells lining epididymis, some are located throughout the duct (principal cells), whereas others are found either exclusively or primarily in specific regions (narrow cells), those cell types are present in most mammals, including humans and ram [12]. Both functions and structures of epididymal cells are varied dramatically between different segments of epididymis, these differences are reflected in appearance and organization of their secretory apparatus (endoplasmic reticulum, Golgi apparatus) and secretory granules (lysosomes and endosomes) [13]. The ultrastructure and functions of cells lining the epididymis as follows: principal, apical, narrow, clear, and basal and halo cells [14]. The global functions of those cells and their secretion are adapted for protection, maturation, nutritive and all factors that served in maintaining those spermatozoa activity and integrity [15].

2. Materials and Methods

2.1 Ethical Approval

An ethical approval was not necessary since no live animals were used for this research; however, the samples were collected from Alshaálla abattoir located in the North-West of Baghdad.

2.2 Collection and processing of testicular samples

Testicular samples were directly collected after donor rams slaughtered and kept in cool box of 4-8°C to transport to the Lab, as discussed by [16], time elapsed from the period of slaughtered to the place of processing must be controlled to be minimized and not be more prolonged which might affect the specimen's quality which altered the final results. Testicular samples were washed thoroughly with distilled water, clean out from surrounding tissues, and then washed with normal saline (0.09%) containing antibiotics (Fig. 1, 2, 3).

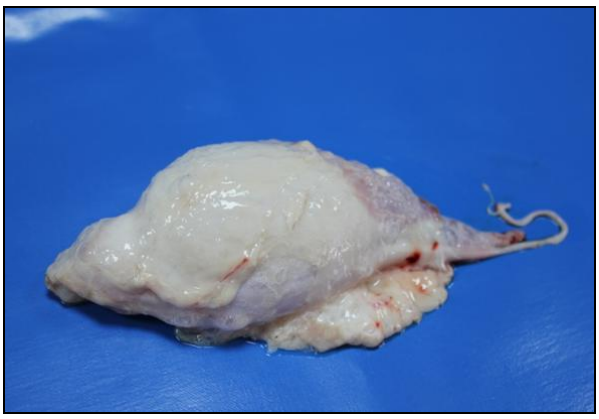

Fig 1: Collected abattoir testicular sample after direct transferred to lab.

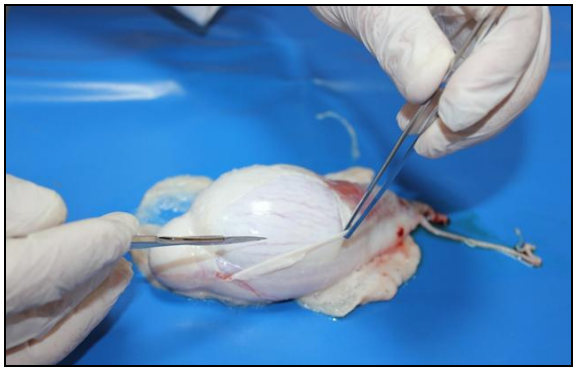

Fig 2: Separations of testicular samples from scrotum and other surrounding tissues

Fig 3: Testicular sample after stripping and surrounding tissues removable

Fig 4: Medium injection inside the cauda of epididymis

Caudae of epididymis injected with medium and separated from entire testicles (Fig.4), then sliced by a surgical blade (Fig.5) and preserved in the refrigerator at 4-8°C for further examinations [17].

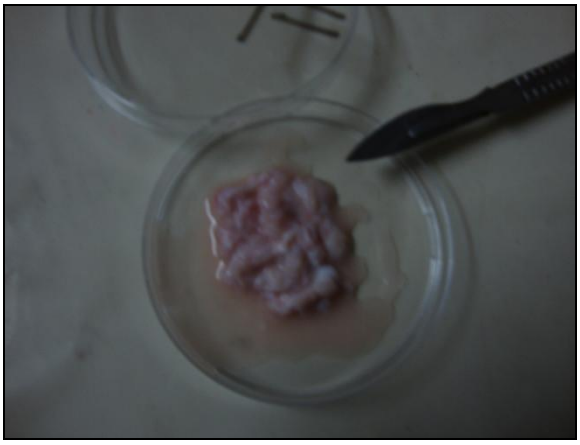

Fig 5: Slicing of cauda by surgical blade after medium injection and separation

2.3 Injectable Media

Media used in this study were Normal saline, Minimum Essential Medium (MEM), Tissue Culture Medium-199 (TCM-199), Glucose, MEM + N. saline, TCM-199 + N.saline and Glucose + N. saline. A plastic syringe connected to gauge 18 needle (Fig.4) contains 8-12 ml of the medium (depending on cauda size) was used. Injection was done by puncturing the cauda by needle; the minimal injection's sites were more and quite suitable [16]. Caudae were chopped or sliced to small pieces by a surgical blade (Fig. 5). Put in dishes and preserved in the refrigerator at 4-8°C for further examinations; all results were recorded [18].

3. Results

Abattoir specimens must be transported directly from site of slaughtered toward the place of processing, duration of time between two evens might affect the final result. Minimal duration of time gave good results.

Tab. (1) and diagram. (1) are shown the effects of prolonged duration of time between donors ram slaughter and specimens processing.

Table 1: The effects of prolonged duration of time between donors ram slaughter and specimens processing

Slaug. Time (hrs.)	Caudal sperms activity (%)	Caudal spermatozoa abnormality	Abnorm. (%)
2	85-90%	Minimal 2nd abnormalities as coiled tail, bent tail and distal droplets	10-12%
6	75-85%	Abnormalities as coiled tail, double tails, distal droplets.	18-24%
24	45%	No. of dead sperms elevated, cells showed head to head, detached tails, local motility	40-48%
36	30%	High number of dead sperm, head to head attachment with no motility,	53-62%
48	20-25%	High number of dead sperms mainly head to head attachment appeared more obviously.	60-78%

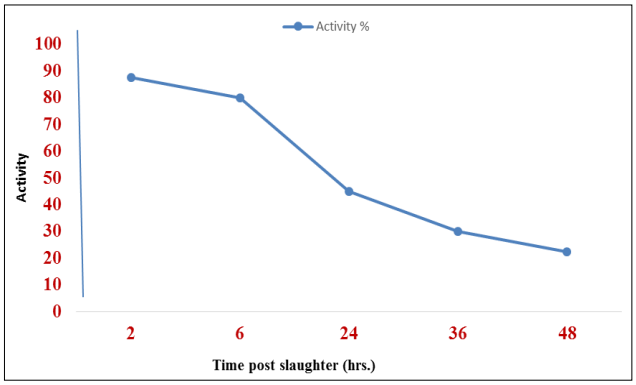

Fig 10: The effect of time elapses from the slaughtering to the Lab processing of the spermatozoa parameters

Motile spermatozoa can be harvested well from cauda of epididymis soon after slaughtered. Cool box temperature used for specimens transferred from the abattoir to the Lab is positively affecting the quality of the specimens when preserved at 4-8ºC, temperature. Cauda samples when preserved at ambient temperature yield a high number of dead spermatozoa with sluggish motility (Table2).

Table 2: The effects of cold preservation of the specimens through transport.

Preservation Temp.	Sperm integrity (live %)	Sperm activity (%)	Sperm abnormality (%)
4-8ºC	85-90%	85%	8-10%
Ambient	45-55%	35-40%	55-60%

Results showed that, it was better to process the specimens directly at it reached to the lab, because it was not so easy to preserve the entire testicular specimens with epididymis even under refrigerator temperature (4-8ºC) for long period, this might be affect the entire testicles as well as the caudae and the final result. Shape, consistency, weight and texture of entire testicles or the caudae both were mainly affected under this prolonged preservation if it was permitted. Table (3) demonstrated this finding and showed the long preservation period effects upon the testicles or the attached caudae which might affect the final result.

Table 3: Effect of cold preservation on testicle with its cauda for periods of time

Time of cold Preservation hr.	Weight of organ (Gs)		Shape of organ		Fluidity of organ		Texture of organ	
	testis	cauda	testis	cauda	testis	cauda	testis	cauda
24	130	8.78	oval	rounded	moist	wet	soft	tender
48	123	8.36	elongated	oval	dryness	rough	flexible	doughy
72	118	8.01	prolonged	oval	hard	Hard	flaccid	doughy
96	106	7.78	extended	almond	harsh	harsh	flabby	masticate

Multiple puncturing of the cauda by 18 gauge needles might damage the cauda tissues and leave many openings, media easily oozing outside the cauda and directly affected the spermatozoa parameters including an increase in the percentage of dead cells toward the living one, decreased motility and increased abnormalities.

Results of using different preservative media revealed no significant and clear effect between them, in concerning to sperms integrity, motility and abnormalities, as shown in tab. (4).

Table 4: Comparison of different media quality in regards to sperm motility, dead or alive for four periods of time.

Medium	24hr *			48h*			72hr*			96hr*		
	Dead %	Alive %	Motile %	Dead %	Alive %	Motile %	Dead %	Alive %	Motile %	Dead %	Alive %	Motile %
N. saline	10±0.57	85±0.85	90±1.73	10±1.15	85±0.57	85±2.30	15±1.70	75±1.10	70±2.30	15±0.57	70±1.73	60±1.15
MEM	10±1.40	85±2.80	85±0.57	13±1.70	78±1.10	80±1.70	26±1.70	70±2.30	70±0.57	28±1.70	65±0.57	60±1.15
TCM	10±0.50	85±1.15	85±2.30	12±0.57	77±1.15	80±1.73	25±0.57	70±1.73	70±1.15	28±0.57	65±2.88	60±1.73
Glucose	12±1.15	80±1.73	75±1.15	18±1.73	70±0.57	70±1.73	30±1.15	60±2.30	60±2.88	35±1.15	55±0.57	50±1.73
MEM+N.S	10±1.15	80±1.73	70±3.46	13±1.15	75±2.30	75±2.88	27±1.15	65±2.30	65±1.73	30±0.75	60±1.73	55±2.88
TCM+N.S	11±1.15	75±1.15	75±2.30	12±0.57	75±2.30	75±1.15	27±1.15	65±1.45	65±0.57	28±1.15	60±1.15	55±1.15
Glucose+N. S	13±1.73	70±4.04	70±1.73	18±0.57	70±2.30	70±1.73	33±1.73	60±2.30	60±2.88	35±2.88	50±2.88	***50***±1.15

LSD for dead=3.1, LSD for alive=4.9, LSD for motile=4.7, Different capital letters denote significant (P<0.05) among different media, and small letters among time.

*Periods of time for 10 samples

**Percentage of each one of the recent parameter

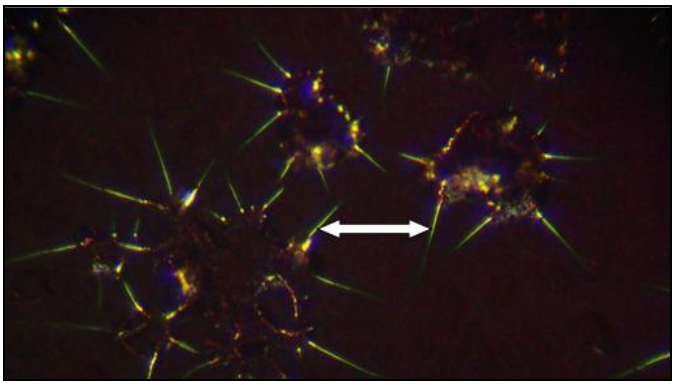

Fig 11: Stained slide field shows alive spermatozoa as head to head agglutination (maturation) white arrow denoted the sperms flagella

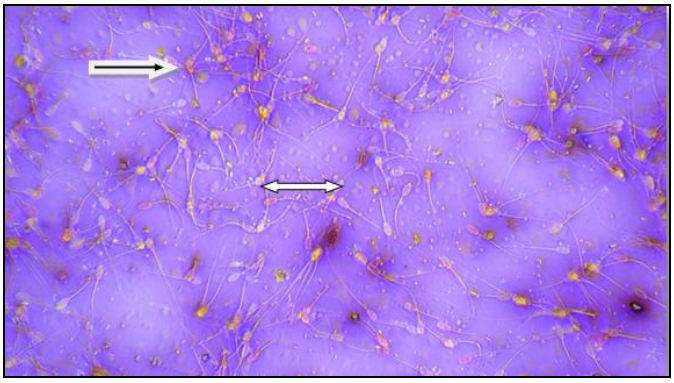

Fig 12: White arrow (bi-point) shows alive caudal spermatozoa, while black one pointed the dead cell.

4. Discussion

4.1 Effect of a period of time elapsed from slaughtering to processing

Effect of duration of time elapsed from specimen's collection to lab processing in regarding to sperms motility, viability and abnormalities, is investigated well.

Lones *et al*., [16] approved that spermatozoa viability or motility is affected as time passed, in which, this is due to decrease the number of live spermatozoa and increase the number of dead that influence the sperm motility.

The ability to preserve abattoir ram testicles for a short period of time study well by Kaabi *et al.* [17] preservation time even at 5°C (refrigerator temperature) affect the spermatozoa activity or integrity. Sperms motility decreased significantly ($P < 0.05$) after24hr, 48hr, and 72hr from time of slaughtering, sperms abnormalities increase after 48 hrs postpartum [21].

4.2 The effects of the different media

Immature spermatozoa due to the effects of cauda environment gain its maturity and acquire motility when they come in contact with epididymal secretion in vivo or (with) preservation media in vitro [19].

Spermatozoa degenerate faster after donor death, but if recovered and preserved in any media (even some hours after death), they maintain its function, mainly when those cells preserved well under suitable conditions and supplied with suitable media [21].

Hamamah and Gatti [22] discussed this event and found that; spermatozoa remain immobile in the lower part of the epididymis and stay-like until ejaculation inside female genital tract or even diluted in an adequate medium.

Among the seven external environments developed by the seven media used in this study, results revealed no significant differences between those media, and according to this result, normal saline medium could be the more accepted one in regards to cheapest, easily manipulated, simply prepared in lab, more stable at ambient temperature and less spoiled ability. According to this results approved by the study (tab.4) we can be declared that; the used of these preserved media were act as diluents for the epididymal secretions of the 5-6 cell types lining the epididymal epithelial segments (principal, basal, apical, narrow, clear and halo), which secret different proteins, H+ ions, hormones and lipids [23] those secretions is the reason for the longevity of caudal spermatozoa.

Al-Timimi [24] reported that the using of different media for caudal spermatozoa collection, maturation and capacitation, the three media used (MEM, TCM-199 and TALP), and concludes that the three previous media gave the same final result without any alignment to each medium but the study favored to use the more practical and cheaper one. Manee *et al*. [25] approved that in his previous study in which the additional supplements to the medium used for improved caudal spermatozoa integrity, all media used for caudal spermatozoa collection have the same physiological effects.

5. Conclusion

According to the results of the present study, the differences were not significant among the different media which was used. Hence the normal saline could be used because it is the cheapest and more practical one. The period among samples collection and lab processing must be limited to minimal period. The more suitable temperature upon specimens transport is between 4-8°C.

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