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Assessment of Caudal Epididymal Sperm of Local Iraqi Ram

KEYWORDS: Epididymis, cauda, testis, spermatozoa, ram.

Wafir M. Saleh
Department of Surgery and Theriogenology, College of Veterinary Medicine, Baghdad University

Ihsan H. AL-TIMIMI
Department of Surgery and Theriogenology, College of Veterinary Medicine, Baghdad University

ABSTRACT *The main objective results of this study was concerned about the time that caudal spermatozoa survived after slaughtered or accidental death, and its ability to fertilize mature ovum to produce good quality embryos in vitro from Iraqi Sheep's specimen obtained from slaughter house from January 2014 to March 2015, all samples were preserved and transporting directly in cool box at 4-8ºC from slaughtering to the time of processing in the Laboratory, the results were conducting according the spermatozoal motility and activities. Those samples were kept in container temperature showed high to considerably elevated motility. The period of time between the slaughtering to the time of investigations in the Laboratory studied well, as the period between the two assignments was kept close or limited to less time consumed, the spermatozoal motility and integrity were elevated. Age of the donor rams, showed topmost results including total spermatozoal accounts and active motility referred to stage or degree of sexual maturity of donor ram testicle samples. Seasonality and testicular orientation (Left and Right) had been investigated also, in which, the left testicle appeared to gain more progressed parameters than the right one. pH of the caudal secretions at time of collections about 6.2, and start to elevate as periods of cold preservations progress to be as 6.9 after cold preservation for 188hr, and this is may be due to equilibration between pH_e and pH_e in related to increased number of dead sperms.. Spermatozoa from detached cauda preserved better from those preserved in entire testicle, Slicing of the cauda after medium injection showed an active motility index when preserved for 24, 48, 72, 96, 120, 144 and 168hr motility index was 85, 85, 85-75, 70-75, 70-75, 65-70 and 60% respectively. Five media were used; normal saline, TCM-199, MEM, dextrose and glucose and mixture of media, the result showed that normal saline from practical view is the cheapest, stable, less spoiled and easily prepared; has the better ability for sperms preservation period. Temperature of injectable medium also investigated, ambient temperature medium showed very good sperms integrity, 85-90% motility and low abnormalities. Spermatozoa aspirated from caudal of lived donor ram showed decreased motility index when preserved for 24, 48 and 96h, motility appeared as 85, 65, and 35% respectively.*

Introduction

Any event that makes male spermatozoa collection or normal mating impossible such as illness, death, castration or injury may terminate Breeding Career or genetics materials of valuable rams. Ram and Buck spermatozoa which are capable for fertilization can be collected by electro ejaculation, or harvested from a region adjust to the testicles called the Epididymis (Shakeri, 2008; Al-Timimi, 2013). 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) were not motile when diluted with saline, but when release from the distal (Cauda) were fully motile and gain the ability of fertilization and 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 (Jones, 1996). Since the number of endangered species is increasing, it is therefore mandatory to develop an effective methodology for their germ cells to be harvested after death and then preserved with acceptable quality (Suhail *et al.*, 2012). Among these importance, people have to develop a protocol to preserve the cauda epididymal spermatozoa that are harvested immediately after Ram slaughter or accidentally died as well as the effect of deferent collecting methods or the preserved temperature or transport distance (Lone,2011).

This study was designed to evaluate the optimum survival methods that keep the caudal spermatozoa a life after slaughter or death of the donor ram.

Materials and Methods

Preparation of samples:

Testicles samples (400) with attached Epididymis from adult rams were collected directly at Al-Shoáalla abattoir after slaughtering, kept in cool box under 4-8 ^{O}C (Lone, 2011) when moved from the place of collection to the laboratory of Theriogenology in the college of veterinary medicine/Baghdad University, in which the process of preparation of spermatozoa samples from undetached epididymis according to effects of: different media used, caudae processing, season, testicular orientation (Left and Right) toward its attachment, the age of ram and period of time elapsed from slaughtering to processing.

Testicle samples after collecting and transporting to the laboratory were washed with distill water firstly, then with normal saline containing penicillin and streptomycin antibiotics, calculate the weight and the size, size of the Cauda, by a small scissor we dissected and separated the epididymis from the entire testicle, long and weight of the epididymis, Caudal weight and size were measured.

Intact Cauda samples were injected with 5-8ml of medium and submitted to the following steps:First aliquot consist of 30 Cauda samples were keep without processing (entire), preserved in the refrigerator for 24hr.,48hr and 72hr, spermatozoa motility and stained smear for dead and alive sperm each time respectively were recorded(Malcotti, 2012), Second aliquot of 30 Cauda samples after an injection with the medium were cut by small sterile scissor to small pieces and kept in petri dishes and preserved under 4-8ºC for 24hr, 48hr and 72hr, same parameters were measured for each period respectively (Foote, R.H., 2000).

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Third aliquot of Cauda samples were injected with 5-8 ml of the medium put in glass Petri dishes and sliced for small pieces by blade, were kept under refrigerating temperature for 24, 48, 72, 96, 120, 144 and 168hr, for each period of time the total motility stained smear (Kaabi, 2003) for dead and alive were examined and recorded. The media used were normal Saline, Minimum Essential Medium, Tissue Culture Medium-199, Glucose (5%) or one partnormal saline with one part of MEM, TCM-199 or glucose.

These types of media were conducted to the slicing method of Cauda preservation; 70 Cauda samples were subjected to study this effect arranged as 10 samples each and preserved at 4-8 ºC for 24, 48, 72 and 96hr, spermatozoa motility were measured for each period stained smear for dead or alive were measured, all results were recorded

Results Discussion

Effects of season:

The effect of season on the testicular, epididymal and caudal parameters of adult ram are mentioned in table (1), in which, seasonality significantly (P<0.05) affect the all the parameters (the ageof donor rams are not involved).

Results of this study mentioned that there is a significant (P<0.05) effect of the season on the testicular weight and size; these effects altered the epididymal parameters, spermatozoa concentration and abnormalities. Milczwski, (2015) found the same results, in which season influenced the testicular parameters (weight and size) and altered the parameters when applied out (–) ve or within (+) ve of the season. Avdi, (2004) mentioned that, testicular volume exhibit seasonal variations, and those variation influenced other parameters as testicular size and sperm concentration. Kridli, (2007) found that, the effects of the seasonal changes among the testicular parameters specially in the Awassi ram breed, in which this breed is more familiar in our country, and this breed appeared to have better sexual performance during the breeding season (autumn), and also mentioned that; ram still had a satisfactory sexual activity during non-breeding season, and for this, abattoir samples in this study cleared this event and showed that sexual activity of Iraqi breed ram still present but for considerable degree. Adriana, (2010) also mentioned that, some breeds of sheep are highly seasonal breeder which influence the reproductive capability and these changes are regulated by photoperiod and the correlation between Melatonin and Testosterone. Moghaddam, (2012) showed that; even with the sever effect of the season on ram fertility which influenced the testicular activity, spermatozoa integrity, motility and seminal quality still can be used in the breeding programs throughout the year.

Table (1) described the effects of seasonality upon parameters (mean± SE) (n=30 of each).

parameters	Out of season*	Within season**
Weight of the Testicle (gm)	96.50 ± 2.00 b	138.60 ± 4.90 a
Size of the Testicle (mm)	42.70 ± 0.50 b	51.72 ± 0.85 a
Size of the Cauda (mm)	17.38 ± 0.27 b	21.98 ± 0.45 a
Length of the Epididymis (cm)	13.04 ± 0.36 b	16.85 ± 0.20 a
Weight of epididymis (gm)	14.08 ± 0.54 b	22.10 ± 0.74 a
Weight of the cauda (gm)	4.64 ± 0.18 b	7.40 ± 0.28 a

Different small letters horizontally denote significant (P<0.05) between out of season and within season.

* Out of season collected samples from 4\ May\2015 to 8\ June\ 2015.

** Within season collected samples from 12\ Dec.\ 2014 to 22\Jan.\2015.

Effect of testicular orientation:

The effect of testicular orientation on ram testicular, epididymal and caudal parameters are mentioned in table (2). Left testicle appeared to gain significantly (P<0.05) more progressed parameters (length of epididymis, size of cauda, size of testicle and weight of testicle) than the right one within and out of breeding season.

Among the results that gained by this study are the variations between the left and right testicles in concerned to the testicular, epididymal and caudal parameters. The left testicles, either that collected out of season or within season, appeared to have progressed parameters than the right in both seasons. The left one being heavier and often larger, this increasing in weight and size also found with the caudal size, epididymal weight, long and caudal weight. Septimus (1975) mentioned that, the two testes are varies much in size in different subjects, and are commonly of unequal size, the left one being more often the larger. Nashikawa. (1960) stated that; both testicles are differ in development, the left one being developed first, and its development more rapid than the right one. Lennox and Logue (1979) found no significant variation between the left and right testes. Siddiqui, (2005) concluded that the main factor influenced the orientation is the age (puberty) more than anything in which the great variation between left and right testes occurred in immature (lamb) ram and the adult.

Table -2 described the effects of orientation upon parameters (mean± SE) (n=30 of each).

Parameter	Left Testicle*		Right Testicle*		LSD
	Out of Season**	Within Season ***	Out of Season **	Within Season ***	
Weight of testicle (gm)	90.80 ± 3.70 b	102.70 ± 5.00 a	85.37 ± 2.00 b	92.50 ± 4.00 b	10.5
Size of testicle (mm)	35.50 ± 0.66 b	40.60 ± 1.20 a	32.40 ± 0.50 c	35.00 ± 1.00 b	2.4
Size of cauda (mm)	14.30 ± 0.33 c	19.10 ± 1.10 a	14.00 ± 0.27 c	16.80 ± 0.50 b	1.5
Long of epididymis (gm)	12.60 ± 0.20 c	15.20 ± 0.60 a	12.40 ± 0.13 c	14.60 ± 0.50 b	1.0
Weight of epididymis (gm)	11.70 ± 0.28 b	15.20 ± 0.50 a	10.80 ± 0.40 b	15.00 ± 0.45 a	1.1
Weight of cauda (gm)	3.60 ± 0.14 b	5.13 ± 0.23 a	3.80 ± 0.20 b	4.80 ± 0.20 a	0.5

Different small letters horizontally denote significant (P<0.05) among left and right and out and within season.

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*Orientation is made in regarding to the inside emerging of the Vas deferens.
** Within season collected samples from 12\ Dec.\ 2014 to 22\Jan.\2015.

Effects of the caudal pH:
At the time of collection the means of lived spermatozoa was about 90% and the pH was about 6.2 to 6.5 and starts to elevate slightly as the time of cold preservation progress to reach about 6.7 to 6.9 after 136hrs and reduction of viability less than 50% as demonstrated in the diagram (1).

Results showed that there was a tendency to acidic medium of the caudal secretion even when slightly elevated as the time of cold preservation passed and this was due to (may be) the effect of the cold preservation or the increasing number of dead cells or may be changing in the biochemistry of the secretion. Florman *et al.* (1989) mentioned that, there is a slow increase in pH_e (external pHe or pHi of inside the spermatozoa) of may be due to **Ca^{2+}, Na^+, K^+** ions interactions and this could be interpreted by recruitment of spermatozoa undergo acrosomal reaction, since the acrosomal vesicle represent an acidic compartment of the spermatozoa (Florman *et al.*, 1995). This compartment lowers the pH_i of the cells, and when this vesicle loses its containment and pH when equilibrates with the pH_e, an increase in sperm pH_i occurred. Spermatozoa pH_i (internal pH) and pH_e (external pH of the surrounding) regulation has not been fully investigated yet, and the value of pH_imainly dependent on value of pH_e in regarding to sperms membrane permeable to protons with a little effect of **Na^+, K^+ or K^+** and **Na^+,** or need further research (Hamamah, 1998).

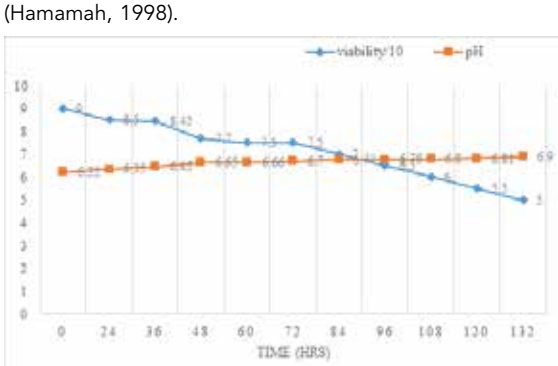

Diagram (1): Shows the effect of cauda pH of on sperm viability.

Effect of the age of the donor ram on caudal spermatozoa integrity and validity:
The result showed that the more sexually matured donor ram is the more spermatozoa parameters revealed, these parameters are; spermatozoa concentration, abnormalities and validity which was 30% at 4 month aged ram and reached 80-85% at 8-14 months old rams, the testicular weight was clearly elevated from 64.7gm at 4 month aged ram to 238.5 gm at 14 month aged ram and can be regarded as the key point (Diagram 2 and Table 3).

The result showed that the donor ram age significantly **(P<0.05)** affect the parameters, as the age of the donor ram proceed which reflects the increasing in the testicular weight, this testicular weight gains affect the sperms parameters (motility, dead and alive percentage), in which, as the age is progressed sperm motility is improved as well as the a live sperms increased. Oyeyemi (2012) found the same result on his study which concerned the testicular and epididymal parameters. Al-Kawmani, (2014) also found that the donor ram age affected positively on the testicular and spermatozoa parameters, spermatozoa concentration extrusive increased with the age of donor ram.

Table (3) showed the effect of age of donor ram upon testicular weight, sperm motility and viability.

Age of donor ram (month)	Wt. of the testicle (gm)	Motility after slaughter (%)	Lived sperm after slaughter (hrs.)
4	64.7	30%	24-36
6	85.5	65%	76
8	105.5	80-85%	96
10	136.3	80-85%	136
12	218.7	80-85%	144
14	238,5	80-85%	168

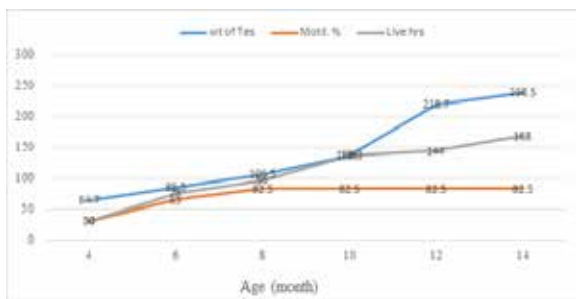

Diagram 2:showed the effect of age of donor ram upon testicular weight, sperm motility and viability.

Effects of period of time elapsed from slaughtering to processing:
Time consumed from the period of slaughtering of the donor ram to the period of processing affect the parameters in which the directly moved specimens to the laboratory gave the best results we in regarding to the spermatozoa motility ranged between 85-90% and fewer abnormalities, the motility decreased to 20-25% when processing done 48hrs after slaughtering (Table 4).

The results revealed that; this period is affect directly the sperms motility, viability and abnormalities. The sperms viability or motility is affected as time passed, in which, this is due to increase number of alive sperms and increase number of dead sperms that influence the sperms motility, also the 2nd sperms abnormalities elevated as the time go on. Shakeri, (2008) found the same results when evaluate the sperms parameters, and showed that sperms motility decreased significantly ($p<0.05$) after 48hr from time of slaughtering and the following hours. Sperms abnormalities increase after 48hrs postpartum. Mir, (2012) also mentioned the same parameters especially after 24hrs from the slaughtering.

Table (4) shows the effect of time elapses from the slaughtering to the Lab. processing on the spermatozoa parameters.

Time after slaughtering (hrs.)	Caudal spermatozoa activity (%)	Caudal spermatozoa abnormality
2	85-90%	Minimal 2nd abnormalities as coiled tail, bent tail and distal droplets
6	75-85%	Abnormalities as coiled tail, double tails, distal droplets.

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24	45%	Number of dead spermatozoa elevated, cells showed head to head attachment, detached tails, local motility
36	30%	High number of dead sperm, head to head attachment with no motility,
48	20-25%	The entire field showed high number of dead sperms mainly head to head attachment appeared more obviously.

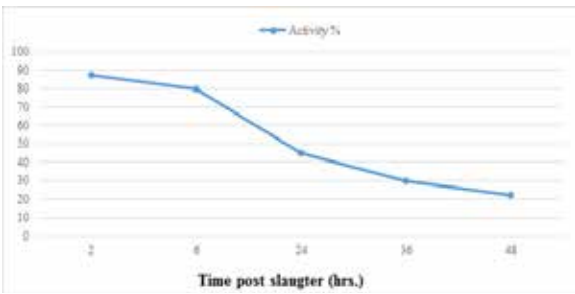

Diagram 3: shows the effect of time elapses from the slaughtering to the Lab. processing on the spermatozoa parameters.

Effect of cold preservation on the caudal spermatozoa quality:
Motile spermatozoa can be harvested well from cauda of epididymis soon after slaughtered and when preserved at 4-8°C, temperature of cool box is positively affect the quality of the specimens' .Cauda samples when preserved at ambient temperature yield a high number of dead spermatozoa with sluggish motility for the rest small number (Table 5).

Tab. (5) demonstrated the effects of cold preservation of the specimens through transport.

Preservation Temp.	Sperm integrity	Sperm activity	Sperm abnormality
4-8 °C	High number of alive sperms.	Moderate to highly active.	Fewer cells with distal droplets, bent or coiled tail.
Ambient	High number of dead sperms.	Sluggish to immotile sperms.	Sperms with distal droplet more prominent, detached head, bent or coiled tails, more sperms appeared as chunk of cells.

The effects of the detachment and attached cauda upon spermatozoa parameters:
Results showed that, it was not so easy to preserve the entire testicular specimens with the epididymis even under cold temperature (4-8°C) for long period, or for each examination time (24hrs). The processes of medium re-injection were spoiled and damage the caudae, and this directly affected the spermatozoa parameters including an increase in the percentage of dead cells toward the alive one, decreased motility and increased abnormalities. Testicles with its cauda attached under refrigeration and when kept for long time started to lose its shape, fluidity and texture and this was directly affecting its content (Table 6).

As the main subject of this study is the caudal spermatozoa and its parameters, the results showed that; the step that try to preserved the whole entire testicle with the epididymis still attached at (RT 4-8°C) for 24hr, 48hr, 96hr, parameters as testicular and caudal weight and size decreased respectively, sperms integrity a and motility decreased respectively, spermatozoa abnormalities increased respectively. Continuously and repeated process of re-injection and aspiration inside the cauda seriously damage the organ and spoil it. Kaabi, M. (2003) found that; a good protocol for postpartum caudal spermatozoa collection is obtained when preserved the cauda at 5°C for periods of time. Lone, FA. (2011) mentioned that; the quality of ram caudal spermatozoa postpartum (not the whole testicle) do not deteriorate for long time.

Tab (6): Effect of cold preservation on testicle with its cauda for periods of time

Time of cold preservation	Weight of organ (gm)		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

The effects of the caudae processing upon parameters:

Results of caudae processing (entire, cutting and slicing) had been well utilized and exercised after detachment from the whole testicle samples, in which:

The entire caudae after medium injection gave satisfied result concerning the spermatozoa index (integrity, motility and abnormalities), for the 1st, 2nd and 3rd attempts (85, 65 and 50% respectively) then declined rapidly to (20%), each attempts need 24hrs apart, medium after injection and re-injection, and, when kept in the becker medium ooze from the cauda outside and that kept the spermatozoa out of its caudal milieu. Dead spermatozoa elevated and the specimens spoiled.

Results of the cutting of the caudae specimens by scissor in Petri dishes after medium injection showed that, the rapid decrease in spermatozoa motility with an elevation in dead number and abnormalities via the time of examination (24hrs apart for 3 days), motility decreased rapidly as (70, 20 and 0-5% respectively). Spoiling of the specimens is obvious that may interfere with spermatozoa index.

Slicing the cauda samples by a scalpel blade in a glass P. dishes after medium injection gave a good results in regarding to spermatozoa parameters and for 1st, 2nd, 3rd, 4th,5th, 6th and 7th day (24, 48, 72, 96,120,144 and 168hrs time elapsed respectively), in which, the motility percentage values were; 85, 85, 85-75, 70-75, 70-75, 70-65 and 60% respectively, some samples still gained its activity for a period of 188hrs post slaughtering (Table 7).

The results obtained by this study concluded that; the slicing method of the cauda yields staying active motility spermatozoa for prolonged period of time against the moderate (entire or aspiration) to humble (cutting) results. Results also showed that the slicing method as it keeps the spermatozoa activity with acceptable degree for prolonged period of time; it yields a decreased sperms abnormality toward the other two methods (cutting and entire), and this is (may be) due to the effects of the epididymal secretions that was mixed with the injectable medium upon ap-

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plying. Rodrigues, (2012) by his study on the ram seminal plasma found that; ram seminal plasma contained proteins which have been associated with several aspects of sperm functions such as; sperm capacitation, protection and activation, this fluid proteins may be shad from the sperm membrane(Thimon *et al.,* 2005) . Seminal plasma may also contained enzymes, hormones, fatty acids and other chemical materials (Souza *et al.,* 2011); all these were diluted with injectable medium and give rise to this good prognosis of the slicing method. Majeed, (2011) found the aspiration (entire) method is the method that yields good and better results. Suhail, (2011) mentioned the slicing (incision) method was the more practical method that yield more and considerable caudal spermatozoa good parameters.

Tab. (7) showed the motility index* of sperms of cauda harvested after post slaughtering periods using different methods of collection.

Processing	Time (hrs.)						
	24	48	72	96	120	144	168
Entire	85%	75%	50%	20%	0.0	0.0	0.0
Cutting	70%	20%	0-5%	0.0	0.0	0.0	0.0
Slicing	85%	85%	85%	75%	70-75%	70-75%	70-65%

*The motility index is regarded as an index for the good health spermatozoa samples.

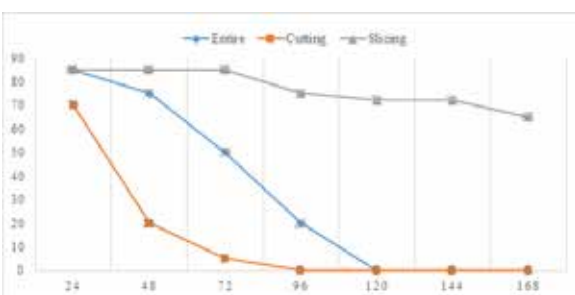

Diagram 4; showed the motility index* of sperms of cauda harvested after post slaughtering periods using different methods of collection.

Effects of different media on spermatozoa parameters:
Results of using different media (table 8) revealed no significant effect between them (MEM, TCM-199, normal saline or Glucose), in concerning to the sperms integrity, motility and abnormalities, but normal saline medium is considered the more suitable one as regarding to a cheapest, easily manipulate, simply prepared in the laboratory, and artlessly in preserved at room temperature (ambient temperature) without spoiling. Stained slides showed the slightly related percentage of dead and alive spermatozoa for each type of media and for those periods of time mentioned (24, 48, 72 and 96hrs).

The cauda epididymis of live animals provide a suitable environment for immature spermatozoa to become mature and acquire motility, in which, spermatozoa remain in quiescent state until gain motility when they come in contact with an external environment as seminal fluid or preservation media (Suhail. 2012). Spermatozoa within the body of dead animal degenerate faster, but if those spermatozoa recovered and preserved in some media, even many hours after death, they still remain function (Sankai, 2001).

Among the 4 external environments made by the 4 media used by this study, the results revealed no significant differences in using those media as spermatozoa preservative media after death. The results also showed that the normal saline medium could be the more accepted one in regarding to the cheapest, easily manipulated, simply prepared in Lab and more stable at ambient temperature.

The results of this study declared that; the used of these preserved media were act as diluents for the epididymal secretions which secreted from the 5-6 cell types lining the epididymal segments (principal, basal, apical, narrow, clear and halo), which secret different proteins, H+ ions, hormones and lipids (Arrotéia, 2012). These secretions are the reason of the long life staying of caudal spermatozoa.

Tab. (8) showed the effects of different media in slicing caudae preservation.

Time (hrs.)	Sperm Parameters % **	Type of Media used			
		Normal Saline	MEM	TCM	Glucose 5%
24	Dead	10±0.57	10±1.40	10±0.50	12±1.15
	Alive	85±0.85	85±2.80	85±1.15	80±1.73
	Motile	90±1.73	85±0.57	85±2.30	75±1.15
48	Dead	10±1.15	13±1.70	12±0.57	18±1.73
	Alive	85±0.57	78±1.10	77±1.15	70±0.57
	Motile	85±2.30	80±1.70	80±1.73	70±1.73
72	Dead	15±1.70	26±1.70	25±0.57	30±1.15
	Alive	75±1.10	70±2.30	70±1.73	60±2.30
	Motile	70±2.30	70±0.57	70±1.15	60±2.88
96	Dead	15±0.57	28±1.70	28±0.57	35±1.15
	Alive	70±1.73	65±0.57	65±2.88	55±0.57
	Motile	65±1.15	60±1.15	60±1.73	50±1.73

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
***Table showed close related values of each one of the parameters in regarding to these periods of time apart.

Effects of medium temperature upon caudal spermatozoa parameters:
Temperature of the medium applied for cauda injections prior to slicing of caudaplayed a great effect upon spermatozoa parameters and for the first injection, result showed that medium hold at ambient temperature gave an excellent results in regarding to the spermatozoa integrity, motility and abnormalities against the refrigerator one (4-8°C) , for this reason and followed the previous step, normal saline medium can be well tolerated the effect of the ambient temperature without spoiling, oppositely if compared with the other media in which the ambient temperature may spoiled them (Table 9).

The process of caudal spermatozoa examination after medium injection is terminating by slicing of the cauda using scalple blade, the caudal mulluei components are mainly proteins, hormones and minerals. Robaire *et al,* (2006) mentioned that; the cauda luminal fluid contained some proteins, some of these, bound to the spermatozoa and affect spermatozoal functions; some facilitate the binding of the spermatozoa to the ZP, some stayed with the

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epididymal lumen, some may be integrated into the membrane of the spermatozoa because of their hydrophobic properties (as GPI anchored proteins), or because of the proteolysis of their carboxyl-terminal region (Gatti *et al*, 2004) .By the previous phenomenon this study found that; a slight elevated in the medium temperature (room temperature) may save some of these proteins or maintained a suitable environment for these proteins to exist their functions.

Table (9) described the effects of medium temperature upon sperms parameters;

Medium temperature	Sperm integrity	Sperm motility %	Sperm abnormality
Refrigerator temperature (4-8°C)	Moderate-poor	60-65	Moderately to elevated dead sperms, coiled or bent flagella, sluggish motility, distal cytoplasmic droplets, and whiplashed movement.
Ambient temperature (25°C)	Very good - good	85-90	Fewer to minimum dead cells, some with distal droplets related to the immaturity, forward movements normal cells shape (head, tail)

Collection of caudal spermatozoa from donor alive ram: Result reveals that, the cauda is the main spermatozoa reservoir and with considerable and aseptic steps, the process of spermatozoa aspiration is done without complication. Action of 3-4ml Xylazin gives a good Tranquilization to the donor ram, which facilitates the whole aspiration process without complications or serious damage to the site of injection (cauda). Cleaning, disinfecting, anesthetizing the site of injection, use the suitable needle gauge connected to a plastic syringe with warm medium improved the results. 2.2 × 10^9 sperms sample concentration, distal cytoplasmic droplets, bent or coiled tail, forward movement (at time of collection), and 85% motility. This declined when preserved 4-8°C for 24, 48, 96hr (85, 65 and 35% respectively). Dead sperms elevated as the time of preservation passed.

The comparative value between the spermatozoa samples gained from alive donor ram with that of the abattoir showed that the ability of abattoir samples to harvested more than the alive one as described in table (10).

The result obtained from this study concerning the refrigerator incubation of fresh caudal spermatozoa acquired by aspiration of the injected warm medium showed that; spermatozoa parameters(alive, integrity, abnormalities) decline after preservation, Moore, (1996) try to preserved caudal spermatozoa using a medium containing epididymal epithelium in which it appeared as the more essential factor for spermatozoa. Pamungkas, (2012) mentioned that; there is no significant variation between ejaculated spermatozoa and caudal spermatozoa after aspiration in concerning to the fertilization index, but both have no ability to save its integrity for prolonged time. Formighieri Bertol, (2013) found same result when he trying to evaluate the spermatozoa parameters of caudal and ejaculated one under 5°C preservation, he intended to develop a short –term preservation for maintained spermatozoa viability. According to the result study spermatozoa have no ability to survive *in vitro* with no caudal effects.

Tab. (10); the comparative value between dead and alive donor ram samples.

Sperm Parameters	Preserved time of					
	A live Ram sample (hrs.)			Abattoir Ram sample (hrs.)		
	24	48	72	24	48	72
Number of sperm ×10^9\ml	2.2	2.1	1.9	2.2	2.1	2.1
Dead %	15	35	75	11	12.5	18
Live %	85	65	35	90	88.2	83.2
Motility %	87	63	20	85	85	80
Abnormality	11	28	43	11	12.2	13

The integrity (dead and alive) and abnormalities of the samples were described in table (11), there is a considerable effects of season upon this results**.**

Table (11): Described the integrity (alive and dead No. and %) of spermatozoa at different periods of time.

Period of Time (hrs.)	Parameter	Within Season	Out of Season*
24	**Dead**	27 (10.8%)	**32** (**12.8**%)
	Alive	**223** (**89.2**%)	**220** (**88**%)
	Abnormal	Minimal secondary abnormalities, distal droplets (15%), double tails (11%), bent tails (12%), detached tails (0.7%) and head to head agglutination (19%)	Abnormal; Distal droplets (11%), double tails (10%), bent tails (13%), detached head (5%) and head to head agglutination (4%).
48	Dead	**31** (**12.5**%)	**33** (**13.2**%)
	Alive	**220** (**88**%)	**220** (**88**%)
	Abnormal	Abnormal; Distal droplets (13%), detached head (0.5%), double tails (11%) bent tails (10%), head to head agglutination more (22%).	Abnormal; Distal droplets (12%), double tails (10%), bent tails (13%), detached head (6%), head to head agglutination (4%).
72	Dead	**45** (**18**%)	**63** (**25.2**%)
	Alive	**207** (**82.8**%)	**192** (**76.8**%)
	Abnormal	Abnormal; Distal droplets (14.5%), detached head (0.7%), double tails (12%), bent tails (17%), head to head agglutination prominent (32%).	Abnormal; Distal droplets (16%), detached heads (8%), double heads (10%), bent tails (15%), head to head agglutination (10%)
96	Dead	**55** (**22**%)	**65** (**26**%)
	Alive	**198** (**79.2**%)	**186** (**74.4**%)
	Abnormal	Abnormal; Distal droplets (10%), detached head (0.8%), bent tails (17.5%), double tails (11%), head to head agglutination still prominent (33%).	Abnormal; Distal droplet (7%), detached head (6%), bent tail (12.5%), double tails (10%), head to head agglutination (30%)

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120	Dead	63 (25.2%)	72 (28.8%)
	Alive	190 (76%)	178 (71.2%)
	Abnormal	Abnormal; Distal droplets seem to be vanished or decrease(7.8%), detached head (0.8%), bent tails(17%), double heads (9%), head to head agglutination still more prominent (37%).	Abnormal; Distal droplet (7%), detached head (6%), bent tail (10%), double tails (11%), head to head agglutination (30%)
144**	Dead	74 (29.6%)	45 ** (18%)
	Alive	178 (71.2%)	205 (82%)
	Abnormal	Abnormal; distal droplets decreased more (5%), detached head (1.1%), bent tails (21%), double heads (13%), head to head agglutination more prominent (38%).	Abnormal; distal droplets (3%), detached head (1%), bent tails (9%), double heads (8%), head to head agglutination more prominent (38%).
168	Dead	78 (31.2%)	187 (74.8%)
	Alive	175 (70%)	63 (25.2%)
	Abnormal	Abnormal: Distal droplets (5%), detached head (2.2%), bent tails (23%), double heads (11%), head to head agglutination (38%)	Abnormal; Distal droplets (9%), detached head (15%), bent tails (25%), double heads (11%), head to head agglutination (24%)
188	Dead	97 (38.8%)	135 (54%)
	Alive	155 (62%)	115 (46%)
	Abnormal	Abnormal: Distal droplets (5%), detached head (2.1%), bent tails (24%), double heads (12%), head to head agglutination (38%).	Abnormal: Distal droplets (8%), detached head (18%), bent tails (24%), double heads (12%), head to head agglutination (19%).

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