قسم : الولادة وأمراضها والتلقيح الصناعي .
كلية : الطب البيطرى ـ جامعة أسيوط.
رئيس القسم : أ.د. / محمود عبد المحسن النجار.

دراسة عن القطيرات البروتوبلازمية

في الأجزاء المختلفة لمجرى الجهاز التناسلي

وفي السائل المنوى

لعجول الجاموس

أحمد جمعه

لقد تم في هذا البحث دراسة توزيع القيرات البروتوبلازمية الحرة وكذا الحيوانات المنوية ذات القطيرات البروتوبلازمية الامامية والخلفية في الاجزاء المختلفة لمجرى الجهاز التناسلي ، وكذا في القذفة الأولى والقذفة الثانية للسائل المنوى . وقد وجد أن النسبة المئوية للقطيرات البروتوبلازمية الحرة تكون أقل مايمكن (١٠ر١ ٪ ± ٨٣ر٤) في الثلث الامامي من رأس البربخ وتزداد نسبتها المئوية تدريجيا في الأجزاء المتعاقبة من مجرى الجهاز التناسلي حتى تصل الى أعلى نسبة لها (٠٦ر٤٢ ٪ ± ٠٨ر٥٩) في أمبولا الوعاء الناقل .

وقد وجد أن الغالبية العظمى للحيوانات المئوية في الثلث الامامي من رأس البربخ ذات قطيرات بروتوبلازمية أمامية ، أما في الثلث الخلفي من رأس البربخ ، وكذا في كل من جسم وذيل البربخ والوعاء الناقل فتكون الغالبية العظمى من الحيوانات المنوية ذات قطيرات بروتوبلازمية خلفية .

كما أنه وجد بالبحث ـ أيضا ـ أن متوسط النسبة المئوية للقطيرات البروتوبلازمية الحرة في القذفه الأولى لعجول الجاموس هو ٧٦ر٢ ٪ ± ٤٢ر٥٤ ٪ ، بينما تزداد نسبتها المئوية بصورة معنوية في القذفه الثانية لتصل الى ٠٣ر٣ ٪ ± ٤٤ر٦٧ ٪ في المتوسط.

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SPERM PROTOPLASMIC DROPLETS IN THE REPRODUCTIVE TRACT AND EJACULATE OF BUFFALO BULLS

(With 2 tables)

By
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(Received at 3/4/1983)

SUMMARY

The distribution of free protoplasmic droplets and spermatozoa with proximal and distal ones was studied in the different regions of 16 reproductive tracts as well as in the first and second ejaculates of 3 buffalo bulls. The percentage of free protoplasmic droplets was very low in the anterior third of the caput (4.83±1.10%) and it increased gradually in successive parts of reproductive tract till reached its maximal level (59.08±4.06%) in the ampulla. Spermatozoa with proximal droplets were prevalent in the anterior third of the caput, while spermatozoa with distal ones were prevalent in posterior third of cauput, body, tail and vas deferens.

The percentage of free protoplasmic droplets in the first ejaculate was 54.42±2.76% while in the second ejaculate it was significantly increased to 67.44±3.03%. A significant increas in the percentage of spermatozoa with distal droplets was also recorded in the second ejaculate.

INTRODUCTION

The protoplasmic droplet was first described by RETZIUS (1909) as a feature of testicular and epididymal spermatozoa of many species. In the testis, at the end of spermateliosis, each sperm cell exhibits a proximal protoplasmic droplet at the neck region (GRESSON and ZLOTNIK, 1945 & 1948, RAO and BERRY, 1949 and BLOOM and NICANDER, 1961). As the spermatozoon passes through the epididymis, there is a shift in its position from a proximal droplet in the caput to a distal droplet in the cauda, wherease in the ampulla, most of the droplets are detached from the sperm cells (BRANTON and SALISBURY, 1947; RAO and HART, 1948; BIALY and SMITH, 1958 and PAUFLER and FOOTE, 1968). The presence of more than 2-3% of the spermatozoa with proximal droplet in the ejaculate of bull is considered to indicate sperm immaturity and spermatogenic disturbances (LAGERLOF, 1934 and 1936).

As far as the free protoplasmic droplets are considered, DOTT and DINGLE (1968), HARRISON and WHITE (1972) and WEITZE (1976) studied their occurance and enzymatic activities in the ejaculates of bull, ram and pig. According to these authors, the protoplasmic drooplets are suggested to contain high levels of glycolytic, lysosomal and glutamic oxaloacetic transaminase activities. The free protoplasmic droplets were described by HASSAN (1979), using electronic microscope to be rounded bodies of about 2 microns diameter, containing many membranous vesicles and lamellae. Moreover, the same author studied their significant influence on stimulating the motility and metabolic activity of washed bull spermatozoa. Recently, OSMAN (1981) reported the presence

of a significant correlation between the free droplets concentration and fructos utilization in the ejaculate of bull.

From the available literatures, the free protoplasmic droplets appeared to receive little attention especially with regard to their distribution in the reproductive tract of animals. Therefore, the present work aimed to study the incidence of free protoplasmic droplets in the reproductive tract and ejaculates of the buffalo bulls. Moreover, the spermatozoa with proximal and distal droplets were also considered.

MATERIAL and METHODS

The reproductive tract of 16 healthy buffalo bulls were obtained from a local slaughterhouse. The epididymis, vas deferens and ampullae were carefully separated. The epididymis was then devided into caput (anterior and posterior third), corpus and cauda. Several incisions were made in each part to allow the escape of its contents into 0.05% Ringer-Formalin solution (HASSAN, BRAUN and LEIDL, 1980). After gentle and thorough mixing, wet preparations were made using a haemocytometer and a phase contrast microscope. The percentages of the free protoplasmic droplets as well as the sperms with proximal and distal droplets were done by counting 200 spermatozoa. The contents of each vas deferens and ampulla were collected by stripping using 0.05% Ringer-formalin as a diluent and wet preparations were made and examined as mentioned before. Each reproductive tract was studied within 1-2 hours after slaughter.

The ejaculates of three healthy buffalo bulls of 2.5-4 years age was studied. Two successive ejaculates were obtained within 20 minutes interval from each bull weekly for a period of 1.5 month using an artificial vagina. Each ejaculate was examined directly after collection for the volume, pH, motility and percentage of alive spermatozoa according to LAING (1979). A haemocytometer and phase contrast microscope were used to count the spermatozoa and the free protophasmic droplets as well as the spermatozoa with the proximal and distal ones (HASSAN et al. 1980). The statistical analysis of the obtained data were carried out according to SNEDECOR and COCHRAN (1967).

RESULTS

The obtained results are shown in tables 1 and 2. From table (1), the results revealed that, the percentage of the free protoplasmic drolets in the anterior third of the caput is very low (4.83±1.10%) when compared with the other parts of the reproductive tract. However, it increases gradually towards the ampulla where it reaches its maximal level (59.08±4.06%). The sperms with the proximal protoplasmic droplet were prevalent in the anterior third of the caput (88.83±2.01%), while sperms with the distal droplet were prevalent in the distal third of the caput, body, tail and vas deferens. In the ampulla 94.08% of the sperms were free from protoplasmic droplets.

From table (2), the percentage of the free droplets increased significantly ($P < 0.01$) from 54.42±2.76% in first ejaculate to 67.44±3.03% in the second. A significant increase in the percentage of sperms with proximal ($P < 0.01$) protoplasmic droplets was recorded in the second ejaculate.

DISCUSSION

The results of the present investigation indicated that the percentage of free droplets in the anterior third of the caput was very low (4.83±1.10%) and it increased gradually in successive parts of reproductive tract till reached its maximal value (59.08±4.06%) in the ampulla where most sperms become free from their droplets. This agrees with RAO HART (1948) who considered the descent of the protoplasmic droplets to be involved with the development of the sperms, when they have reached maturity, the droplet move to the distal position where it is ready to be casted off without particular reference to the location of the sperm in the genital tract.

BIALY and SMITH (1958) explained the releas of the protoplasmic droplet to be due to either a mechanical or a chemical faotor or both operating during the transition from the epididymis into the ampulla or the ejaculate. Theoratically, each sperm should have one protoplasmic droplet; this means that the expected number of free protoplasmic droplets in any part of the genital tract should be equal to the number of the sperms without droplets. The present results indicated that, in the ampulla 35% of the expected free droplets were lost. The percentage of loss of free protoplasmic droplets decreased gradually in the direction of the anterior third of the caput, where it reached its minimal level (3%). Such loss of free protoplasmic droplets could be explained in the light of the work of HASSAN et al. (1981) who found that the period of the storage of semen at 37°C resulted in the disintegration of the free protoplasmic droplet. It seems possible that the loss of the free droplets in the cauda and ampulla is due to their disintegration which might be attributed to storage capacity and the relatively high body temperature. According to the findings of several authors (DOTT and DINGLE, 1968; HARRISON and WHITE, 1972 and WEITZE, 1979) the enzymatic content of the free droplets may play a role in the maturation of spermatozoa during their passage in the ductus epididymis.

The percentage distribution of sperms with protoplasmic drolet in the different regions of the reproductive tract (Table 1) coincide generally with the results of BRANTON and SALISBURY (1947), RAO and HART (1948) and HANCOCK (1955). However, differences between the percentage of values in the present work and those recorded by BIALY and SMITH (1958) and EL-AZAB and OSMAN (1970) may be due mainly to the method of counting where they used stained films. This may cause droplets translocations and disappearance (BIALY and SMITH, 1958 and BLOM, 1943). The counting method in the present work by using fixed wet preparations was proved to be more accurte (HASSAN et al. 1980).

The results of the present work revealed a significant increase in the percentage of free droplets in the second ejaculate ($P < 0.01$) and this agrees with the findings of HASSAN (1979) for cattle bull. It is well known that the quality of the second ejaculate of the bull is better than the first one. HASSAN (1979) found that the addition of isolated free protoplasmic droplets to washed bull spermatozoa caused a significant increase in the sperm motility which accompanied by increase of fructose utilization and lactic acid production, Moreover, OSMAN (1981) proved the presence of significant correlations between the concentration of free droplets in the ejaculate and each of fructolysis, sperm motility, sperm concentration and live sperm percent. It seems possiple that the better quality of the second ejaculate may be due to the significant increase in the concentration of the free protoplasmic droplets in comparison to the first ejaculate.

The significant increase in the percentage of sperms with distal droplets from the first (2.24±0.49%) to the second (4.10±0.60%, $P < 0.01$) ejaculat agrees with the findings of LAGERLOF (1934), GOTZE (1949) and HASSAN (1979) in cattle bull.

ACKNOWLEDGEMENT:

I wish to express my sincere thanks to Prof. Dr. A.M. Osman for his help and continuous interest.

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Table (1): Percentage distribution of free protoplasmic droplets (PT) and spermatozoa with proximal and distal ones in the reproductive tract of buffalo bulls

	Epididymis					
	Head		Body	Tail	Vas deferens	Ampulla
	Anterior third	Posterior third				
Free PT %	4.83±1.10 (0-10)	12.42±1.71 (4-25)	18.42±2.87 (5-40)	24.20±3.54 (9-54)	31.42±2.56 (13-49)	59.08±4.06 (31-77)
Proximal PT %	88.83±2.01 (77-98)	35.50±4.50 (15-61)	8.25±1.04 (3-14)	3.41±0.54 (0-6)	2.08±0.42 (0-5)	1.50±0.42 (0-5)
Distal PT %	3.33±1.16 (0-13)	44.66±5.45 (18-73)	64.33±3.29 (38-83)	58.50±3.97 (28-80)	48.92±3.01 (30-71)	4.42±0.97 (1-10)

Mean ± S.E. n = 16

Table (2): Sperm concentration and percentage distribution of free protoplasmic droplets (PT) and each of proximal and distal ones in first and second ejaculates of buffalo bulls

Creteria	Sperm concentration ($\times 10^3/mm^3$)	Free PT%	Proximal PT%	Distal PT %
Bull A:				
1st Ejaculate	1406.66±117.59	58.61± 3.46 (45.95-69.29)	1.46±0.48 (0.00-3.41)	1.32±0.33 (0.00-2.31)
2nd Ejaculate	1182.00±112.51	66.46± 7.78 (35.06-83.11)	2.03±0.65 (1.35-5.19)	2.44±0.49* (1.23-4.29)
Bull B:				
1st Ejaculate	1145.00±144.78	53.92± 4.22 (40.16-70.09)	1.23±0.44 (0.00-2.65)	2.52±0.72 (0.58-5.77)
2nd Ejaculate	1173.33± 59.76	70.27± 4.09* (52.48-83.89)	2.14±0.36 (0.99-3.60)	5.38±1.13 (2.91-11.19)
Bull C:				
1st Ejaculate	1165.00±169.91	50.74± 5.78 (33.77-79.31)	2.26±0.36 (0.83-3.72)	2.88±1.13 (0.00-8.51)
2nd Ejaculate	1255.00±102.13	65.58± 3.69 (51.53-78.52)	2.44±0.35 (1.34-3.94)	4.47±0.87 (1.74-7.09)
Total :				
1st Ejaculate	1238.88± 88.64	54.42± 2.76	1.65±0.31	2.24±0.49
2nd Ejaculate	1203.44± 54.04	67.44± 3.03**	2.20±0.26*	4.10±0.60**

* P/ 0.05
** P/ 0.01

n = 6, Mean ± S.E.
t = test, percentages after arc. sin. transformations