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A POSSIBLE NEW APPROACH FOR DETERMINING THE POST-MORTEM INTERVAL

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In many medico-legal cases there is a need to know the approximate time of death. It has been pointed out by Schleyer[1] that the time of death is important in cases such as: unwitnessed fatal traffic accidents, homicides, and some suicides. A solution of a homicide case may depend upon a fairly accurate determination of the time of death, which is particularly important when several successive crimes have been committed by the same individual. Therefore, it is essential that the determination of the time elapsed since death be determined and such procedures justify extensive laboratory procedures.

A current review of the literature indicates at least nine laboratory methods which show promise for use in medico-legal cases. Biochemical methods include the measurement of the variation in the amino nitrogen, non-protein nitrogen, creatine, ammonia, and inorganic phosphorus in the plasma and/or the cerebrospinal fluid following death.

Four physiological methods which have shown promise are the measurement of the faradic excitability of muscles, the pupillary reactions upon injection of pilocarpine or homatropine into the anterior chamber, the *in vitro* clotting of the fluid blood from peripheral veins, and the formation of an idiomuscular contraction by mechanical irritation. There are, of course, other methods which are not considered to be laboratory techniques. The above methods are of no value after the thirtieth hour post-mortem, which in many cases offers the greatest difficulty in assessing the time of death.

According to Schleyer[1] an exact chronology of post-mortem changes do not and cannot be expected to exist. In all probability no single method will ever provide a dependable range of accuracy, yet there can be little doubt that application of several tests may furnish the examiner with the degree of certainty which is required for medico-legal determinations.

When circulation ceases, tissue anoxia results in necrosis followed by decomposition. The time in which cells can continue to metabolize following somatic death varies according to the tissue and may be erratic. Laboratory tests presently used to determine the post-mortem interval depend upon the time it takes for certain tissues to cease to function, or upon chemical or physiological changes resulting from the necrosis. These tests in current use are only reliable up to the thirtieth hour post-mortem. Therefore, to make estimations of the post-mortem interval beyond the thirtieth hour, it becomes necessary to look for some biological entities which can live for extended periods of time without blood or oxygen supply.

Ham[2] reports that spermatozoa are anaerobic and can survive anaerobically on carbohydrates by fermentation. During fertilization they live for a number of hours without oxygen, utilizing the carbohydrates in the female genital tract for energy. According to Guyton,[3] sperm can be stored and maintain their fertility in the genital ducts for as long as 42 days. Earlier reviews by Marshall[4] state that the spermatozoa of the bull may survive for as long as twelve days within the removed testicle if the latter be kept at a temperature a little above the freezing point. No documented information concerning the use of spermatozoa for estimating the post-mortem interval could be found.

[1] Schleyer, *Determination of the Time of Death in the Early Post-mortem Interval*, in 2 METHODS OF FORENSIC SCIENCE 253–93 (F. Lundquist ed. 1963).

[2] A. W. HAM, HISTOLOGY 815 (1957).

[3] A. C. GUYTON, TEXTBOOK OF MEDICAL PHYSIOLOGY 1121 (1966).

[4] H. A. MARSHALL, PHYSIOLOGY OF REPRODUCTION 177–78, 650.

A possible new approach for estimating the post-mortem interval beyond the thirtieth hour is suggested by sperm survival in the genital tract. A preliminary investigation was, therefore, conducted to determine the concept that sperm viability after death may be utilized for estimating the post-mortem interval.

Methods

Two sets of bovine testes were made available to us by the local slaughter houses. The scrotum and vas deferens had been removed leaving the testes and epididymes enclosed in the tunica. The head, body, and tail of the epididymes were completely intact.

One set of testes had been in storage for several days (168 hours) and the other set had been removed from a bull which had been dead only two hours. Both sets had been maintained at an average temperature of 38°F after slaughter. The testes were placed in plastic bags containing a small amount of normal saline and stored in the laboratory at 38°F. Sperm samples were immediately taken from each specimen by making a small slit in the wall of the epididymis. A sample was taken of the epididymal fluid which extruded from the incision onto a clean glass slide. Two slides were prepared for each specimen. The epididymal fluid on one slide was mixed with a drop of egg yoke semen extender[5] which had been stored at the same temperature as the specimens, since changes in temperature result in a high mortality rate to the sperm. A cover slip was placed over the preparation to prevent drying and the motility of the sperm estimated. The specimen for the second slide was collected in a similar manner and prepared according to Sorensen and Miller.[6] A drop of Schaffer's and Almquist's[7] differential stain was added to the epididymal fluid and thoroughly mixed. A thin smear was prepared by placing another clean slide on it and drawing it along with a gentle motion. The slide was then dried over the flame of a Bunsen burner. The differential stain is a staining mixture of 1% eosin B and 4% aniline blue dissolved in M/8 phosphate buffer having a pH of 7.2. This stain is used to differentiate between live and dead sperm; the heads of dead sperm are stained light blue, while live sperm remain unstained.

[5] E. J. Perry, The Artificial Insemination of Farm Animals 116 (1952).

[6] A. M. Sorensen & L. Miller, Animal Reproduction, A Laboratory Manual.

[7] Shaffer & Almquist, *Relation of the Eosin-Aniline Blue Staining Method to the Quality of Bull Semen*, 32 J. Dairy Sci. 723 (1949).

On the following day one epididymis from each set was placed in a constant temperature oven at 90°F. One specimen was 24 hours old, and the other was 192 hours old. Microscopic observations were continued every twenty-four hours through the fourth day on the refrigerated samples. After 24 hours, the tissue decomposition of the incubated specimens became so advanced that samples could no longer be taken. It should be noted that the seven day old sample was manifesting signs of decomposition before it was incubated. At the termination of the study the older refrigerated sample was 288 hours (12 days) old.

Results

Initial microscopic observations showed numerous motile sperm in both the 168 hours and 2 hour-old specimens. The sperm manifested the same normal pattern of motility as seen in a healthy bovine semen sample.

According to the differential staining technique, which distinguishes between live and dead sperm, approximately 80% of the sperm in both the 168 and 2 hour-old refrigerated specimens were alive. This percentage slowly declined in the older specimen to an average of forty percent at 240 hours. At the final observation after 288 hours about 50% of the sperm were fully stained and the remaining 50% were partially stained. This would indicate that 50% of the sperm had died recently and the cell membrane was in the initial stages of becoming permeable to the dye.

There was a steady decline in viable sperm in the 2-hour old refrigerated specimen. According to the observed motility and staining results it was estimated that about 50% of the sperm were still alive at 120 hours post-mortem. The extent of decomposition made it impossible to get representative samples from the incubated specimens after 24 hours. Both incubated specimens exhibited viable sperm when sampled at 24 hours but percentage estimates were not made.

The sperm appeared normal in all slides studied. Figure 1 shows the sperm in the samples taken at 48 hours following a 24-hour incubation period at 90°F, and figure 2 shows a representative sample taken at 288 hours at 38°F, respectively. Numerous well-formed spermatozoa were present in all slides studied.

Discussion

The results of this study show that bull spermatozoa, and presumably those of other mammals in-

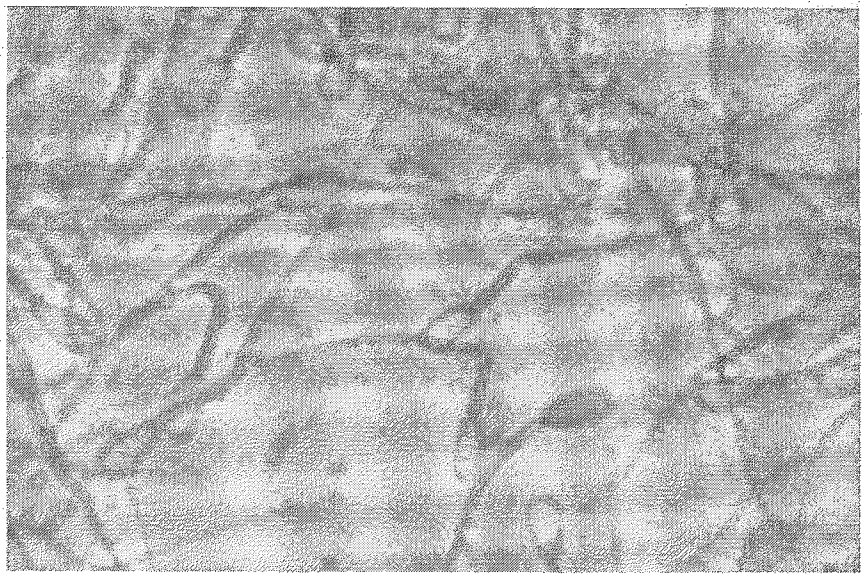

FIGURE 1
Spermatozoa taken from an epididymis refrigerated for 24 hours at 38°F followed by an incubation period of 24 hours at 90°F.

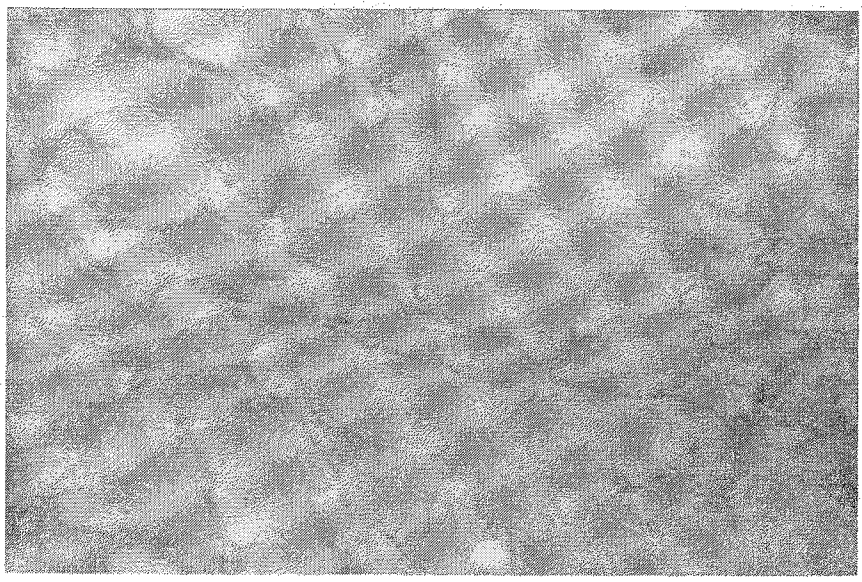

FIGURE 2
Spermatozoa taken from an epididymis stored for 264 hours at 38°F.

cluding the human, will remain viable in the epididymis for an extended period of time after death. The post-mortem viability of the sperm is temperature dependent. Increased temperature will increase the metabolic rate of spermatozoa and shorten their life span. It will also increase the rate of decomposition of the surrounding tissue. The combination of those two factors contribute to the increased death rate of the spermatozoa which might be directly an indicator of the post-mortem interval.

This preliminary study has provoked interest in exploring the feasibility of utilizing spermatozoa or related material for estimating the interval after death beyond thirty hours. Possibly some of the other methods which have been used for estimating

the quality of semen in animal science could be utilized for estimating the age of the sperm following death, and, correlated with the ambient conditions, give a dependable method of estimating the time following death of the individual.

These methods measure (1) the duration of spermatozoa motility at a standard low temperature, (2) the glycolytic power of spermatozoa, (3) oxygen consumption of spermatozoa and, (4) the resistance of spermatozoa to temperature shock or a 1% sodium chloride solution. Perhaps a modification of the Methylene Blue Reduction Technique of Beck and Salisbury which gives an index of the concentration and activity of the sperm could be used here.

Methods should be developed for correlating the viability, motility, death rate and perhaps morphological and histochemical changes in the epididymal fluid with time and temperature.

In its simplest form this method could be used to roughly estimate the post-mortem interval, if the length of time that epididymal sperm will remain alive or motile under a certain set of conditions were known. For example, if it were known that sperm are motile for 10 days in the epididymis of a body which has remained at a temperature varying between 32° and 38°F following known time of death, it would be possible to estimate whether a body in which the time of death was unknown had been dead greater than or less than ten days.

If this method proves feasible it would probably have its greatest value in the colder climates or during the winter. Much more research is required to establish relationships of viability of spermatozoa with temperature and other ambient conditions. The results presented in this paper indicate that sperm remains viable and motile during a post-mortem interval where existing tests are unreliable.