GOAT SEMEN COLLECTION AND PROCESSING

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INTRODUCTION

The object of this manual is to bring together the goat semen collection and processing techniques that are available for use by the Artificial Insemination (AI) industry. Its purpose is to provide a reference source of operation techniques that will help semen production staff establish and/ or improve semen quality and quantity.

It is important to remember that there are many different techniques which will give satisfactory semen quality for AI, but not all will give maximum reproduction efficiency. Not all semen processors are in agreement as to what is the best technique to use. What "works" in their hands may be the result of biological variations in the buck and even in environmental and management conditions at the point of collection.

There are many factors that will contribute to successful semen collection and processing techniques including nutrition, animal housing, processor training, etc. These subjects will be dealt with to a limited degree in the text. Other subjects such as reproductive physiology are covered briefly, and the readers are referred to other manuals and books on the subject listed under *Recommended Readings*.

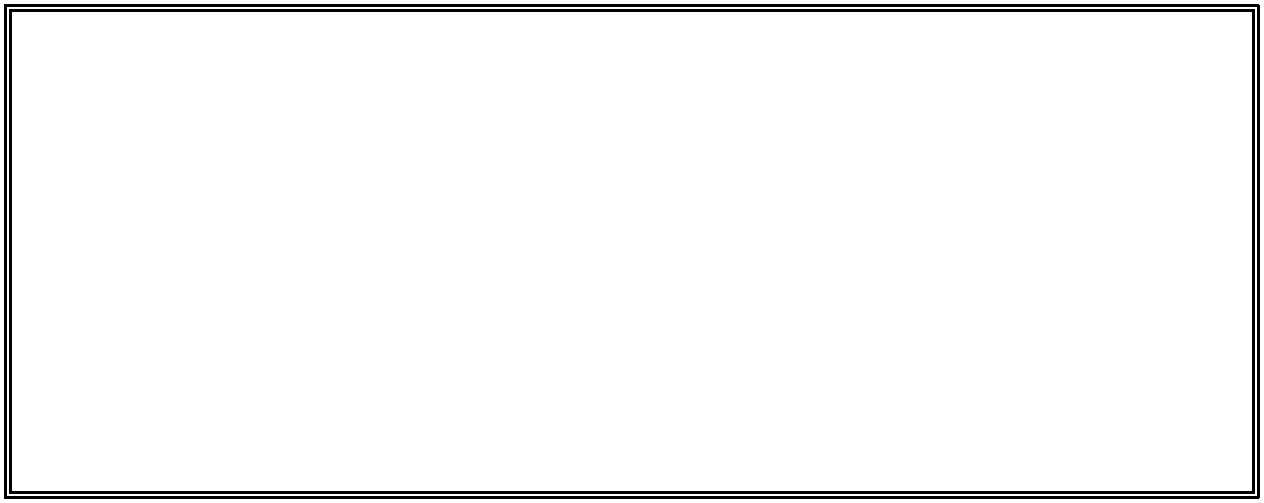
I. Semen Production and Collection

A. The Development of the Sperm Producing System

The onset of the production of spermatozoa represent only the final step in a series of complex changes that govern their number and properties. These events start prenatally when the

primordial germ cells migrate from the germinal crest and come to lie in the presumptive gonads sometime before sexual differentiation. In the fetus and young male, the gonocytes are contained inside the seminiferous tubules. These multiply and after birth give spermatogonia. The quantitative efficiency of spermatogenesis depends to a great extent in the manner in which these divisions take place. The cells originating from the last spermatogonial division are the primary spermatocytes. Meiotic division of these result in the production of daughter cells, the secondary spermatocytes. The secondary spermatocytes divide and form spermatids which undergo a complex series of changes (spermiogenesis) resulting in the spermatozoa. The quality of the spermatozoa produced depends to a great extent on this complex metamorphosis which occurs in the seminiferous epithelium but undergoes completion in the epididymis. The various germ cells are located in the seminiferous epithelium, whose structure is maintained by the Sertoli (nurse) cells (see Figs. 1 and 2). The two major functions of the testis include androgen synthesis by the interstitial cells (Leydig) and sperm production within the seminiferous tubules. Although these two functions are separated anatomically, intercellular communications exist between them.

FIGURE 1: Hypothalamus



The postnatal development of the male reproductive tract has received some attention, primarily in the bull and ram. Puberty is commonly defined as being the point in time when the male first produces sufficient sperm to impregnate a female. In many of the domestic species it is practically defined when an ejaculate is obtained containing 50 million sperm of which 10% are motile. The pubertal period is associated with rapid testicular growth, changes in LH secretory pattern, a gradual increase in blood testosterone, and the initiation of spermatogenesis. The hypothalamus is believed to play a key role in initiating puberty because the pituitary gland, gonads, and steroid-dependent target tissues are each competent and ready to respond to their respective tropic hormones prior to puberty. In the boar, bull and ram, and probably the buck, puberty appears to be the culmination of a continuous and lengthy process of endocrine changes which begin shortly after birth. The most remarkable early change is an increase in the frequency of pulsatile discharge of LH. This is followed by altered testicular steroidogenesis, increased circulating levels of testosterone, differentiation of Sertoli cells and the onset of spermatogenesis.

It has been proposed that puberty occurs when an animal becomes desensitized to the feedback inhibition imposed on the hypothalamic-pituitary complex by gonadal steroids. Presumably, the allows an increase in GnRH discharge and a greater response by the pituitary gland to GnRH.

Spermatogenic Cycle

The spermatogenic cycle begins with a stem cell or A-type spermatogonia, which provides the starting point of a spermatogenic series. The duration of spermatogenesis, that is from the time it

takes an A-type spermatogia to end up as spermatozoa in the buck is probably very similar to what has been reported for rams, namely 47 days.

The impuberal testis is under pituitary control. The multiplication and differentiation of supporting cells are gonadotropin dependent. In lambs, the testis weight and the number of supporting cells which decrease after hypophysectomy are maintained after injection of LH. FSH has a synergistic action with LH. Testosterone is ineffective. The mitotic activity of the gonocytes is more pituitary independent but differentiation into spermatogonia, giving rise to spermatogenic activity with the subsequent appearance of the primary spermatocytes, is under the influence of pituitary gonadotropins. In adult rams (and probably bucks) LH, FSH and prolactin are secreted in pulsatile patterns with peaks of testosterone secretion following 30 to 60 minutes after each plasma LH peak.

Onset of sperm crop production in bucks is apparently breed dependent. It is known that sperm cells begin to be produced in high concentrations (2×10^9/ml) in 1.0 ml ejaculates with very good wave motion in Alpine bucks at 4 to 8 months of age, at 7 to 9 months in Boer goat bucks and considerably later in the Damascus breed.

B. Semen Collection Technique

The methods commonly used for collection of ram and buck semen are the artificial vagina (AV) and the electroejaculator (EE). The most commonly used and easiest procedure is the use of the AV. The AV method is painless, quicker and does not stress the animal at all. The AV resembles a car radiator hose and is about six inches in length. It has an inner rubber liner (containing water at a temperature of 100°F) placed between the liner and the hose. The warmer water simulates the vagina of a doe. A latex rubber collection cone is placed in the AV and a graduated collection tube

is placed on the end of the cone.

The buck is collected for semen evaluation or processing by allowing him to mount a doe in heat, another buck, or a wether. The usual procedure is to use a teaser doe that is in heat. This can be a natural heat or one induced by a prostaglandin product. A doe in heat usually stands better for a buck than a wether or another buck. She emits a smell when in heat that causes the buck to give a better ejaculate. The doe is usually tied or held and the buck allowed to go through his courting behavior. The buck is allowed a few false mounts, then the person with the AV collects the ejaculate by directing the penis into the AV. The test tube containing the ejaculate should be protected from direct sunlight and cold temperatures.

Electroejaculators come in a variety of shapes and sizes and are used when animals are not trained for AV collection, are physically unable to mount, or a suitable mount is not available. Most workers have obtained a greater volume of semen but of lower concentration of spermatozoa with EE that with an AV. Some animals do not respond well to the electrical stimulus, especially if a second or third collection is desired. Further, there is some danger of contamination of the semen sample with urine. Greater concentrations of sodium and potassium were found in both the sperm and seminal plasma obtained by EE. The increased volume of seminal plasma obtained by EE appears to reduce the resistance of sperm to cold shock and decreases the post-thaw survival rate of frozen semen.

Limited comparisons of fertility showed that the conception rate at first service was 17% higher when semen was collected with an AV. However, other reports have not found a significant difference in fertility with semen collected by these two different methods. Recent advances in the anatomical and physiological knowledge of the male reproductive tract may lead to EE techniques

which permit acquisition of normal sperm to seminal plasma ratios in the ejaculate.

II. Factors Influencing the Output of Sperm

In addition to the role the method of collection plays as discussed above, there are a number of other factors which influences the size and sperm content of the ejaculate.

A. Seasonal Factors

In bucks and rams, changes in day length influenced testicular activity by modifying the release of GnRH and thus the gonadotropins. In seasonal breeders, the volume of the ejaculate is high in the breeding season and decreases in the non-breeding season (Fig. 20). Sperm concentration follows an opposite trend. However, no seasonal variations in total sperm numbers per ejaculate are reported. When the volume increases or decreases, the changes of volume are mainly due to changes in quantities of fluids secreted by the accessory glands and the epididymis which are androgen dependent. The androgen receptors in target organs need a preliminary preparation in which prolactin has been implicated. In turn, prolactin secretion is associated with daily photoperiod of long duration (i.e., spring and summer days at northern latitudes). The latter knowledge was brought about by studies in rat and sheep, but may well apply to the male goat in which blood plasma prolactin levels rise in the spring to culminate in summer and drastically decline in the fall, while the androgen abruptly rise takes place in the middle of the Summer to culminate in the Fall and decline in the middle of winter.

Figure 20. Seasonal Variations in Volume and Sperm Concentration of Ejaculates (m ± sd).

B. Age, Breed, and Within Breed Differences

As in other species of livestock, there are breed differences with respect to age of puberty, ejaculate volumes, sperm concentrations, and total sperm per ejaculate. In early maturing breeds such as the Alpine, sperm numbers per ejaculate in the first breeding season represent only 60% of those collected in the second breeding year, which are characteristic of adult sperm production capabilities (Graph 3). In addition to semen production between breeds, there are differences also observed in the amount of sperm ejaculated by animals of the same age and breed. Animals may be classified and ranked for breeding replacement potential early after puberty (i.e., mean of 15 ejaculates over an eight week period). The resulting ranking appears to remain the same in the first few breeding seasons (Graph 4).

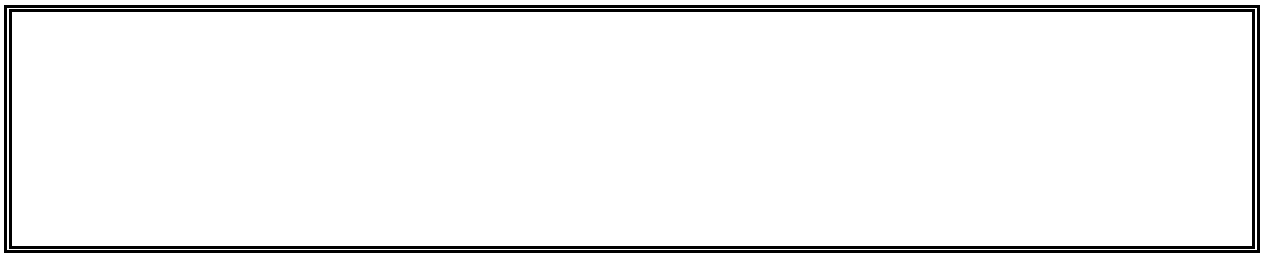
Delayed puberty can be the result of breed, within-breed genetics, poor pre-pubertal nutrition, diseases, parasitism, month of birth, and injury. Animals not producing high quality semen by the time they are 12 months of age become a concern and should be considered candidates for culling.

Old age arrives in bucks at different ages, depending on health, environment, and use. Most animals of 8 to 10 years of age appear to have adequate sperm numbers but an increase in abnormalities. The best prediction of how long a buck will live and produce good viable semen, while not 100% accurate, is to evaluate blood lines. This requires, of course, many individual observations within each group. With the onset of old age, there are bucks who have episodes of excellent semen production which lasts a period of weeks and then goes into another period of poor quality semen from which he may or may not recover. The reason for these episodes are unknown and difficult to predict.

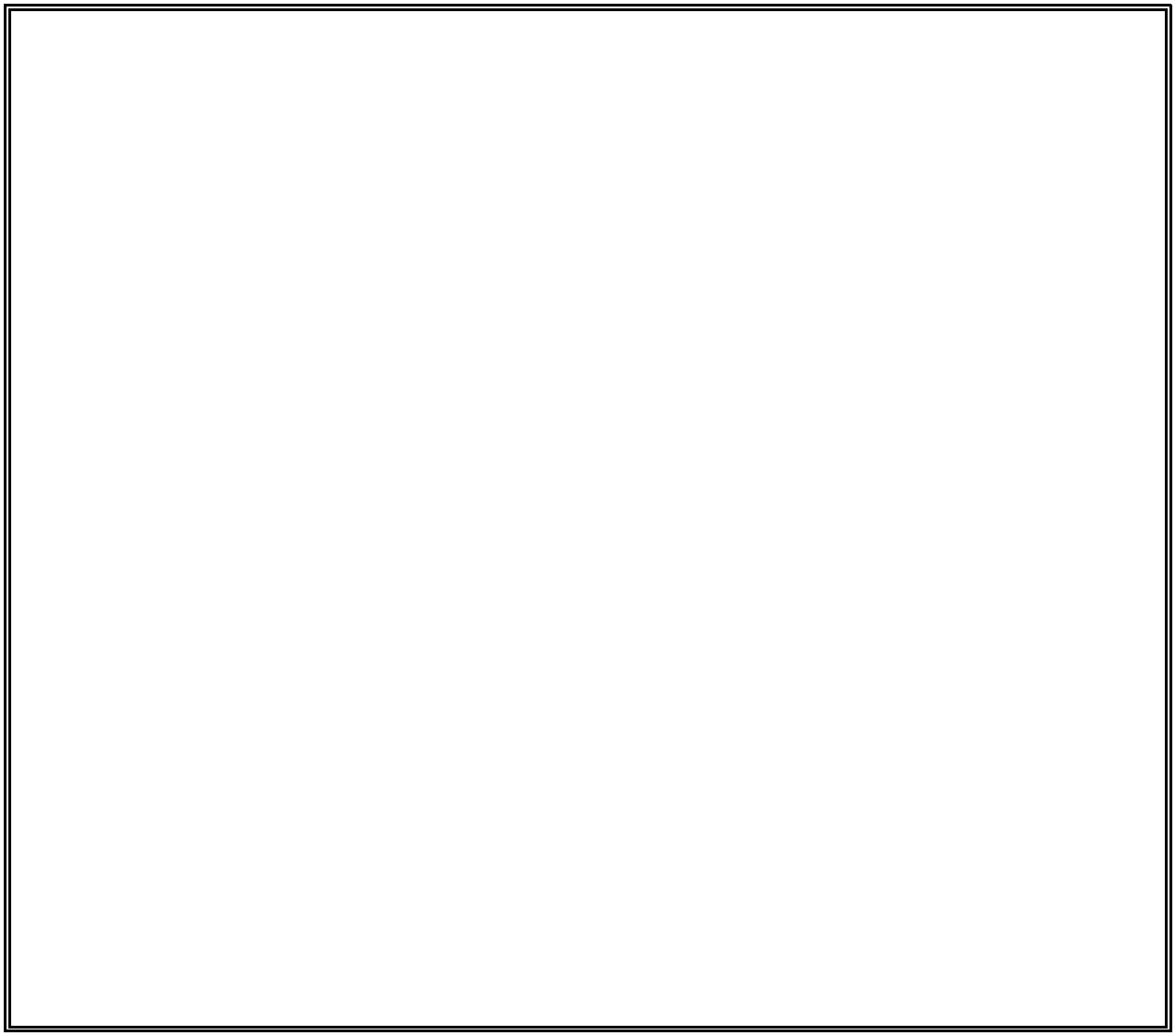
C. Unknown Factors Influencing Sperm Output

The amount of good quality semen is decreased by hyperthermia associated with high ambient temperatures with high relative humidity or fever. Low levels of energy intake, poor quality forage and lack of particular nutrients all contribute to poor ejaculate quality. Precise knowledge as to the most practical semen collection frequency to obtain optimal sperm quality and quantity is lacking. Most processors and buck stud operators work on an individual by individual basis.

GRAPH 3: TOTAL NUMBERS OF SPERMATOZOA ...



GRAPH 4: MALE GOATS OF TWO BREEDS RANKED ACCORDING TO SPERM PRODUCTION



The resorption of sperm is weak in the epididymis with most loss occurring in the urine or by masturbation. The epididymal sperm reserves available daily for ejaculation are readily depleted with approximately 62% of the potential daily sperm collection being obtained in the first two ejaculates. Epididymal replenishment is fairly rapid and collections twice per day at 24 hr intervals have been practiced in well-managed facilities. In adult Alpine males ejaculated once per day during the breeding season, weekly production of up to 25 billion spermatozoa per animal have been attained.

Even with our ability to supposedly package enough viable and morphologically normal cells in a straw or ampule, there remains factors of semen that contribute to fertility (or lack thereof) that are not well understood. It has been found in many bull studs that bulls with high semen quality credentials give lower than desirable fertility. Conversely, some ejaculations of "apparent" poor visual quality readily settle animals. These unknowns might range from subtle differences in the DNA makeup to handling and inseminating interacting with the semen available. Recent development of tests to evaluate the integrity of the internal nucleic acid component may shed light on fertility availability.

Subjective evaluation of semen has been the one method commonly used out of necessity, because no other alternatives were available. Unfortunately, this approach is often prone to observer bias and error. To eliminate this bias, a number of computerized semen analyzers have recently been introduced onto the market. Any system of this type must rely on image size, shape, and/or grey scale characteristics to distinguish individual spermatozoa and differentiate them from debris that may be present in the field of view. Such discrimination is not 100% accurate. Even if a system were very accurate and precise in its tracking and detection, the objective data obtained might not be

predictive of sperm fertility. However, these systems have evolved to the point where reliable, accurate data systems have evolved to the point where reliable, accurate data on cell counts, concentrations, number of motile cells, % motility, mean linearity, straight line velocity, curvilinear velocity, and lateral head displacement can be generated (Fig. 5). Also, some systems are able to track and plot individual sperm cells for several seconds (Fig. 6). It is now possible to determine the effects of processing treatments on these parameters. Coupling this "high technology" assessment of semen quality, with a bioassay assessing fertility, would greatly strengthen the assessment of a buck's reproductive value. Such a bioassay may be the use of the zona-free hamster ova penetration test developed by Yanagimachii in 1976. He found that capacitated human sperm cells were capable of penetrating hamster vitellin (zona-free ova) by removing the zona pellucidae and that the fertility of the male was correlated with the level of penetration.

AUTOMATED SEMEN ANALYSIS REPORT

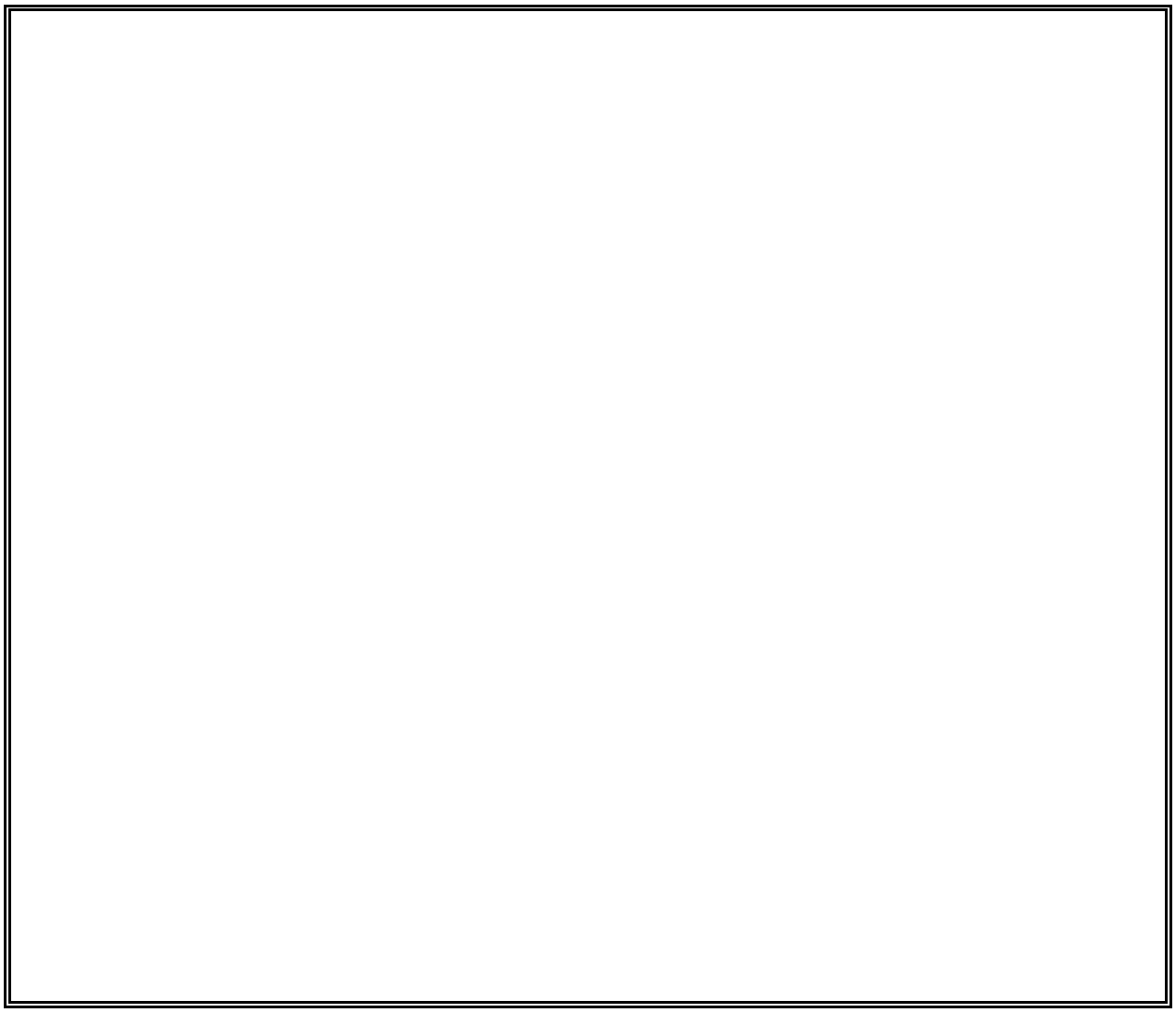
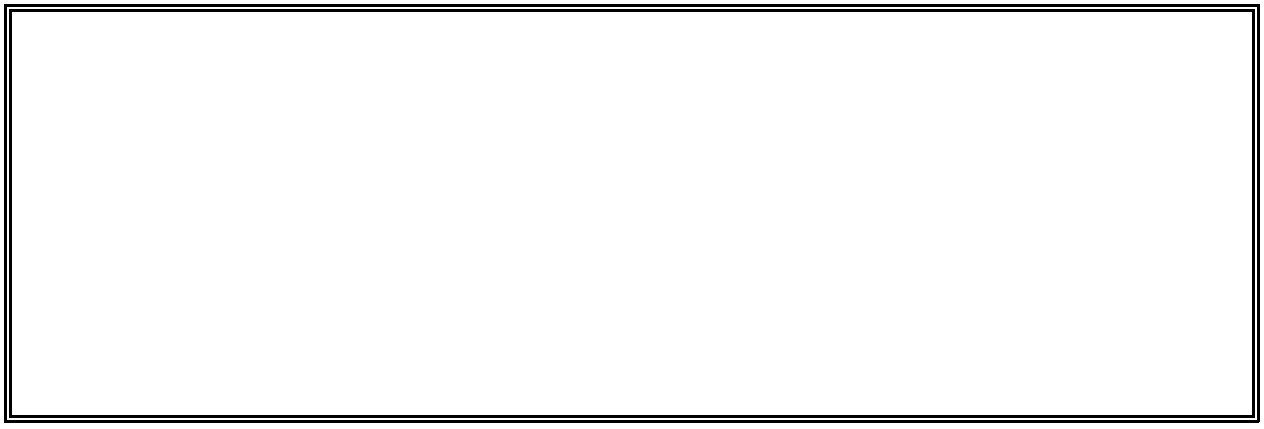


FIGURE 6: GOAT SPERM SWIMMING PATTERNS: 10 SECONDS DURATION



III. Sperm Cell Preservation

Spermatozoa, by nature, are catabolic cells. This means that in the metabolic processes they deteriorate as they use stored energy. With time, they age and slowly die off until all cells are immotile and incapable of fertilization. Likewise, semen subjected to adverse environments will irreversibly deteriorate and become useless. Because sperm have little or no anabolic or healing capacity, anybody working with any stage of semen processing must be attuned to the potentially harmful environments in which semen is placed. The main objective in semen processing is to minimize the catabolism of semen so that the quality of thawed semen is similar to that which was originally ejaculated. By doing this, semen can be packaged so that more than the minimum threshold number of viable cells are available in the oviducts.

There are almost as many suggested freezing procedures and extender formulations as there are researchers studying AI in goats. The long list of extenders include nearly every imaginable kind

of medium from cow's milk, to coconut milk, to tomato juice, to various exotic mineral waters. I will only discuss a few extenders, the relative importance of these components, and some controversial ideas about semen preservation. Areas of importance that can contribute to how well semen survives processing and thawing are length of holding time at warm (room) temperatures after collection, cooling rates to 35°F eventually to -320°F; extender composition, antibiotics used, and method of thawing. These factors act individually or interact with each other. To find the current balance of conditions under varied laboratory and/or field conditions to reach our desired quality levels is the challenge for every processor.

A. Liquid Stored Semen

Generally, when semen is extended and stored above 0°C, sperm cell survival is of a few days duration and sperm fertilizing ability cannot be preserved for more than a few days. Sperm cell survival is generally longer than fertilizing capacity and the former cannot be used as an indication of the latter.

B. Long-Term Storage

Most, if not all, semen is preserved in a frozen state. In order to accomplish this goal, suitable extenders must be used. An ideal extender has to meet certain requirements: a) provide nutrients as a source of energy; b) contain constituents that provide protection against the harmful effects of cooling and freezing; c) provide a buffer to prevent shifts in pH as lactic acid is formed; d) maintain the proper osmotic pressure and electrolyte balance, e) contain antibiotics that inhibit bacterial growth, f) substantially increase the volume of semen so that multiple inseminations can be performed; and g) provide an environment in which metabolic activities of the sperm can continue.

Most extenders for liquid or frozen semen have been either egg yolk or milk or a combination

of the two as a basic ingredient. The primary benefit derived from fresh egg yolk is protection of the sperm cell against cold shock by yolk lipoprotein and lecithin. The milk protein, casein, has been established as the agent responsible for prevention of cold shock.

Milk Extenders

Goat semen has been diluted in saline, sodium citrate or skim milk. Of these extenders, only skim milk heated at 95°C for 10 minutes satisfactorily preserved the fertilizing capacity of goat spermatozoa.

Cow's milk has been used as an extender in routine artificial insemination of goats with good fertility results. A skim milk glucose-glycerol extender has also been used to freeze goat semen.

Egg Yolk Extenders

There are two schools of thought regarding the use of egg yolk as a component of a goat semen extender. Semen diluted with egg yolk has resulted in acceptable conception rates for some workers. In 1,007 goats inseminated with sodium citrate glucose-yolk-glycerol extended semen, conception rates were 60 and 92% with frozen thawed and fresh semen, respectively.

Other investigators believe that egg yolk is toxic to the goat spermatozoa. An enzyme (phosphotidase) produced by the bulbo-urethral glands of the male goat catalyses the hydrolysis of lecithins in egg yolk to fatty acids and lysolecithins, which are toxic to the spermatozoa. The presence of phosphotidase in the seminal plasma of the goat means that media containing egg yolk cannot be used for semen extension.

Seminal plasma has been blamed as a limiting factor of goat semen freezability and fertility. Washing goat spermatozoa in a physiological solution improved the percentage of motile sperm in fresh ejaculates. Post-thaw motility of spermatozoa was also increased.

Semen Extension Rate

The objective of extending semen is to provide an appropriate volume of inseminate with a sufficient number of sperm to give high fertility without wasting sperm.

Sperm concentration of the ejaculate varies from 2 to 6.5 x 10^9 sperm/ml. Highest fertility in ewes was reported for sperm suspension frozen at a concentration of 666 x 10^6 sperm/ml. Another worker inseminated goats with 1 x 10^9 sperm/ml and reported fertility.

Semen of rams and bucks is routinely extended immediately after collection at 37°C and cooled slowly to prevent cold shock, resulting in sperm damage. Lipoprotein and lecithins, found in egg yolk, and milk, provide protection from cold shock when added to the semen before cooling. The optimum cooling rate is one that prevents cold shock but is fast enough to maintain healthy, viable sperm cells. After extension, semen is cooled at 5°C at approximately 0.5°C/min.

Glycerol is added to extenders to protect sperm against the detrimental effects of freezing. It has been suggested that the increase in intracellular salt concentration due to the removal of water from the cell during ice formation is a major cause of sperm damage during freezing.

The protective action of cryo-protectants, such as glycerol is largely attributed to their salt "buffering" capacity; thus, electrolytic damage as the water freezes out is minimized.

The time, temperature, rate of addition, and concentration of glycerol have been studied with many conflicting reports. Some researchers have added the glycerol before cooling (i.e., at 30°C) and others at 4°C. Since cryo-protective activity of glycerol occurs during the crystallization phase, glycerol should be added at 4°C.

The level of glycerol is generally 4-6% in extended ram semen. One worker has used 6-9%, and another added 14% glycerol to freeze goat semen.

Semen equilibration time after addition of glycerol has been studied with conflicting results. However, 3 to 4 hours appears sufficient for ram semen and 1½ to 3 hours equilibration time seems adequate for goat semen. Longer equilibration times does not appear to be detrimental.

The following pages list two of the formulations which appear to work with goat semen. The final procedure presented is the one I have used extensively with good success. I use it because of its simplicity and results. What you use should depend upon availability of materials and satisfactory results.

IV. Semen Extender and Procedure A

Ingredients	Amount	
Skim milk	100	ml
Glycerol	7	ml
Penicillin g potassium	100,000	units
Streptomycin sulfate	100,000	micrograms

NOTE: The above ingredients are used to make 50 ml each of 2 extenders; PART A - non-glycerolated, and PART B - glycerolated.

1. In a double boiler heat the 100 ml of skimmed milk to 95°C (203°F) for 10 minutes.
2. Allow the milk to cool to room temperature and divide it into two portions: 50 ml - Part A and 43 ml Part B.
3. Add 100,000 units of penicillin G and 100,000 micrograms of streptomycin to Part A and mix thoroughly. These antibiotics can be bought from a number of pharmaceutical firms in pre-weighed powdered form. Your veterinarian can probably help you get the antibiotics and figure out how to dilute them to achieve the proper concentrations.
4. Add 7 ml of glycerol to Part B (43 ml of skimmed milk) and mix.

5. Make sure you label the two parts clearly. During the semen processing, if you add the wrong one first, you may kill the sperm. Both parts can be stored in the refrigerator for a day, so you can make up the extender the day before you plan to collect the buck.

V. Semen Washing Solution (Ringer Solution)

Ingredients	Amount to Make	
	100 ml	1 liter
NaCl	0.86 gm	8.6 gm
KCl	0.03 gm	0.3 gm
$CaCl_2.2H_20$	0.033 gm	0.33 gm
H_20 to	100 ml	1000 ml

1. Weigh out the ingredients listed above.
2. Add 50 ml H_2O (or 500 ml of H_2O, if making up a liter) to the powdered ingredients.
3. Mix until all the ingredients are dissolved.
4. Add H_2O to bring the total volume to 100 ml (or 1000 ml, if you are making a liter).

Semen Preparation and Extension

1. Immediately after collection, gently mix the semen with 20 parts of Ringer solution (i.e., if the ejaculate volume is 1.2 ml add 24 ml Ringer).
2. Centrifuge the diluted semen at 1000 g for 10 minutes at room temperature.
3. After centrifugation, pour off or aspirate the supernatant (liquid) - being careful not to disturb the pellet of sperm in the bottom of the tube.
4. Add the same amount of Ringer solution used in Step 1 and carefully re-suspend (re-

dissolve) the pellet by slowly sucking it up into a pipet and letting it flow out 5 to 10 times.

5. Repeat Steps 2 and 3.
6. Add 1 ml of Ringer solution to the sperm pellet and re-suspend it.
7. Take a 0.05 ml sample of the semen and add it to 10 ml of Ringer. This diluted sample is used for concentration determination. If a hemocytometer is used, multiply the count by 10.05×10^6 to find the concentration.
8. Add 2 ml of Extender Part A (non-glycerolated) to the 1 ml of semen. Both extender and semen must be at the same temperature.
9. When the concentration has been determined and the dilution rate calculated, bring the semen up to exactly ½ the total required volume with Extender Part A.
10. Place the tube with the extended semen in a beaker or Erlenmeyer flask to which 100 ml of room temperature water has been added. This volume of water surrounding the semen will ensure the proper cooling rate.
11. Place the semen, with its water jacket, into a refrigerator or cold room adjusted to 4°C (39°F). Also, place the amount of Extender Part B (glycerolated) needed for final extension in the refrigerator. (Equal to the volume of Extender A used).
12. After 1 hour, add Extender Part B to the semen dropwise with a pre-chilled pipet.
13. Package the semen in straws or ampules and seal. Allow the sealed packages to stay in the cold at 4°C (39°F) for 4 hours.

Semen Extender and Procedure B

Semen. Semen samples should be maintained at a constant temperature of 37°C. A 10°C increase above this will increase metabolic rate and decrease life span of sperm. Above 50°C, sperm suffer

irreversible loss of motility within five minutes. Proper semen handling includes the following:

1. Identification of collection vials for each animal: If possible, each vial should be permanently identified for one animal and used for only that animal. This identification should include the number assigned to that animal, and the number of that collection. Use of colored tape for different species of individuals assures greater accuracy.
2. Handling of sperm: Sperm handling must be done with extreme care. This includes time of collection right up until insemination of females. When collected at either cool or warm ambient temperatures, the semen must be insulated at all times. This includes storing collection equipment in incubators set at approximately 37°C. Collection vials should be covered with some type of insulating material to maintain the temperature. After collection, these vials should be transferred to a water bath set at 37°C. If volume is to be recorded directly after collection, a graduated collection vial makes this possible with minimum exposure time.

Semen Extenders: Each type of extender was prepared in a 1000 ml graduated cylinder. All crystalline materials were weighed on a triple-beam scale. All liquid materials were measured out from a 100 ml graduated cylinder. For the TRIS extender, egg yolks were separated from the egg whites. The yolks were then dried on a paper towel, then punctured, and allowed to drain off into the 100 ml graduated cylinder. No membranes or egg whites were allowed to contaminate the liquid yolks. Antibiotics were crystalline powder diluted in a stock solution of 1 million units or ug/ml. They were added to the buffer. Distilled H_2O is then added to make a final volume of 500 ml of extender. After mixing, the extenders were then poured off into 50 ml containers and frozen. Appropriate extenders were then thawed the morning before use. The composition of all extenders is

shown in the following table.

Extending Formulas

Formula	Diluter A (w/o glycerol)	Diluter B (w/glycerol)
Tris Egg-Yolk		
Tris (g)	12.1	12.1
Citric Acid, monohydrate (g)	6.7	6.7
Glucose or Fructose (g)	5.0	5.0
Penicillin (units/ml)	1000.0	1000.0
Streptomycin (ug/ml)	1000.0	1000.0
Glycerol (ml)	---	70.0
Egg Yolk (ml)	100.0	100.0
Distilled H_2O to final volume (ml)	500.0	500.0
Milk-Glucose		
Milk Powder (g) (1% fat)	50.0	50.0
Glucose (g)	0.97	0.97
Penicillin (units/ml)	1000.0	1000.0
Streptomycin (ug/ml)	1000.0	1000.0
Glycerol (ml)	---	70.0
Distilled H_2O to final volume (ml)	500.0	500.0

Semen Evaluation: The volume of a fresh ejaculate was recorded immediately as measured in a calibrated collection tube. After the volume was recorded, the sample was examined for opaqueness and for any contaminants that the sample may contain.

The motility of a semen sample was estimated in a properly diluted specimen so that 100 individual sperm can be seen per field at 400 x under the microscope. Cool slides will reduce motility. Hot slides will kill the sperm. This procedure was used for freshly collected and extended semen.

With a glass capillary tube, a small drop of semen was placed on a pre-warmed slide in a drop of sodium citrate. The two parts were mixed and covered with a cover slip, all the while on a

slide warmer at 37°C. Extended semen was examined directly without dilution, frozen thawed semen was also examined directly. The slide was then placed on a properly set microscope stage incubator at about 37°C. The slides were observed under 400x, and at least ten areas of the smear were used to estimate the percent of motile sperm. All of the slides were estimated to the nearest five percent for total motility. More abnormal sperm were found near the edge of the slide. This was most probably due to cold shock and drying out of the preparations.

Processing Semen:

Goats: Immediately after collection, the volume of the ejaculate was recorded and the semen placed in a water bath at 30°C in order to prevent cold shock. Two sub-samples were taken from the fresh semen, one to estimate initial motility and the other for the purpose of determining concentration of the Spectronic 20. From each ejaculate (250 ml of semen - or, if the total volume of the ejaculate was less than 500 ul) half of the volume was added to each of two 15 ml conical tubes containing 10 ml of Kreb's Ringer Solution at 30°C and mixed by carefully inverting the tubes three times. This solution was then allowed to cool to room temperature (22-24°C) in a rack for 15 minutes. After this period of time, the solution was centrifuged for ten minutes and 600-800 g, after which the supernatant was carefully removed with a pasteur pipette. The packed cells remaining in each conical tube were re-suspended in six ml of non-glycerated extender at room temperature. Milk-based extender or egg-tris extender was added to the tubes. After mixing by careful inversion, the solutions were poured into 25 ml tubes. Each 25 ml tube containing pre-extended semen was placed into a 250 ml Erlenmeyer flask containing 200 ml of water at room temperature, and placed in a cold room at 50°C for two hours. The temperature of the water in the flasks, which indicated the temperature of the pre-extended semen, was checked and recorded every 15 minutes during the two hour cooling

period. At the end of this period, the tubes were removed from the flasks and placed in a rack in the cold room. Two ml of glycerated extender at 50°C were added at intervals for 10 minutes, carefully mixing by inversion after each addition, until a total of six ml had been added. The fully extended semen was then packaged into straws, held at 50°C for equilibration until freezing in liquid nitrogen vapors.

Modified Kreb's Ringer Phosphate Buffer[a]

Ingredients	Millimols	gm/liter
NaCl	120	7.0
KCl	5	0.36
KH_2PO_4	10	1.36
$MgSO_4.7H_2O$	5	1.23
Tris	10	1.21

[a] Supplemented with 0.5% (5 g/l) glucose. Otherwise as described by Garbers, First and Lardy, 1973, Biol. Reprod. 8:589.

Semen Extender and Procedure C

Because of the numerous factors which may be critical in the day-to-day and week-to-week operations of extenders including water quality availability, ingredient variations, technician differences, etc., an attempt was made to locate a commercially available extender which was suitable to use with goat semen. Such a commercially available extender was that available from Reproduction Resources (Hebron, Illinois 60034 USA) which sells a product called continental diluent and is specified for use with cattle and goat semen.

Although the exact formulation has not been revealed, discussions with the salesmen have revealed that it is probably a TRIS-Citrate buffer with glycerin (4-6%) used as a cryo-protectant. This can be obtained by the ½ or full gallon and is apparently stable at room temperature for several months. Using this as a starting point, the following procedure is the one I have personally used

most frequently.

1. On the day that the bucks are to be collected, measure out 250 ml of continental diluent in a graduate cylinder.
2. To this, add 1.0 ml of Linco-Spectin antibiotic (Upjohn Corp., Kalamazoo, MI 49001 USA) and mix. Each ml contains 50 ml Lincomycin HCL and 100 mg Spectromycin sulfate.
3. Add 10% by volume of egg yolk. Make sure the egg white is separated completely and that the yolk membrane is blotted dry before tearing to harvest the yolk. Mix with a gentle swirling motion until mixture appears homogeneous.
4. Pour solution through a funnel fitted with a folded milk filter to remove any clumps.
5. Refrigerate until ready to use. Discard whatever is not used after 24 hours.
6. Just before collecting the buck, take out the extender and warm to 37°C (98°F) and maintain at that temperature.
7. Collect the bucks. Record the volume and remove a 20 ul sample and add it to 8.0 ml of 3% NaCl for semen quantitation. Immediately add 4.5 ml of warmed extender to semen and store at 37°C.
8. Read the sample taken for quantitation on a Bausch and Lomb Spec 20 which has been calibrated for this purpose (See Table IIIB).

TABLE IIIB: Goat Semen Concentration Chart

%T	CONC. ($x10^9$/ml)	%T	CONC. ($x10^9$/ml)	%T	CONC. ($x10^9$/ml)
20	10.16	44	6.52	68	2.87
21	10.01	45	6.37	69	2.72
22	9.87	46	6.21	70	2.57
23	9.71	47	6.06	71	2.41
24	9.56	48	5.91	72	2.26
25	9.40	49	5.76	73	2.11
26	9.25	50	5.61	74	1.96

27	9.10	51	5.45	75	1.81
28	8.95	52	5.30	76	1.66
29	8.80	53	5.15	77	1.50
30	8.64	54	5.00	78	1.35
31	8.49	55	4.85	79	1.20
32	8.34	56	4.69	80	1.05
33	8.19	57	4.54	81	0.90
34	8.04	58	4.39	82	0.74
35	7.89	59	4.24	83	0.59
36	7.73	60	4.09	84	0.44
37	7.58	61	3.93	85	0.29
38	7.43	62	3.78	86	0.14
39	7.28	63	3.63	87	*Negligible*
40	7.13	64	3.48		
41	6.97	65	3.33		
42	6.82	66	3.17		
43	6.67	67	3.02		

* This regression will estimate semen concentration only when a Bausch and Lomb Spec 20 is used. Wavelength should be set at 630, and a 1:400 dilution should be made with 3% NaCl.

9. Multiply the concentration off the chart by the total ejaculate volume to get total sperm count. Divide this number by final concentration per ml to your desire and this will give you final extended volume.
10. Centrifuge for 15-20 minutes at 800-1000 rpm at room temperature or until you achieve a soft semen pack and a clear supernatant solution.
11. Carefully aspirate off supernatant solution.
12. Re-suspend sperm pellet to final extended volume.
13. Place in a 500 ml beaker containing room temperature water and place in a refrigerator (35-40°F) for 6 - 12 hours.
14. Mix gently and pour into filling dish which is kept cool in a walk-in refrigerator or in a

portable floor cooler.

NOTE: Extended semen and all materials which come in contact with the semen must now be at the <u>same</u> refrigerated temperature.

15. Fill straws (½ cc) and seal. Place on straw freezing rack.
16. Place liquid nitrogen into a styrofoam chest so that it is 5 cm deep. Let it sit for 5 - 10 minutes covered.
17. Place straw freezing rack containing filled straws into freezing chest so it is suspended 2 inches (5 cm) above the level of the liquid nitrogen. Cover chest, leave for 12-15 mins.
18. Remove cover and quickly dump straws into liquid nitrogen.
19. Remove a straw and thaw in 95° water for 1 minute. Open and evaluate.
20. If semen is acceptable, cane up straws and put into storage tanks.

V. Semen Evaluation:

With the increase in the use of AI in the goat, semen evaluation of the buck has assumed considerable importance. This is a particularly important process when buck semen is intended for distribution. The goal of semen quality evaluation is to assess the products fertilizing ability. The majority of the studies concerned with semen evaluation, in the past, has been performed with bull and ram semen and these procedures have been extended to buck semen.

Extensive research has been conducted over the last 50 years to identify parameters that measure sperm fertility. Currently, there is no single endpoint that can be used to definitively evaluate a semen samples' fertilizability. The parameters are generally used in

combination to evaluate an ejaculate. As indicated previously, the combined use of computerized semen analysis systems and zona-free hamster ova penetration assays may prove to be a powerful set of tools but the costs are currently prohibitive for most commercial production facilities. The following is a list of the more common ejaculate characteristics used for evaluation.

1. Concentration (millions of sperm/ml)
2. Progressive motility (%)
3. Acrosome morphology (% intact acrosomes)
4. Volume (ml)
5. Tail morphology (% abnormal)
6. Mass motility (%)
7. Ejaculate color

8 pH

Why do we bother to perform these evaluations? Even though fertility cannot be accurately predicted, the lack of it can. Deviations from the norm is usually a good predictor of low fertility. Our objective in evaluating semen quality should be to identify those samples that have traits pointing to the probability of below normal fertility and therefore discard them. An extension of this thought is to discard those males that continuously produce poor quality semen.

In general, semen quality can be divided into two categories: a) the number of sperm cells harvested and b) the viability of the collected sperm cells. Quantification of sperm cells can be readily determined with the proper equipment and training. It is simply the product of

the ejaculate volume and the sperm concentration.

The color and consistency are often, but not always, a function of concentration. With some practice, an experienced person can "guess" the sperm cell concentration with some degree of accuracy just by looking at an ejaculate. Of course, such guesses are just an approximation and should never be used for serious evaluation purposes.

Assessing the viability is a much more difficult task. It takes training and usually some sophisticated laboratory equipment. The problem is compounded by the fact that reproductive physiologists still have not precisely defined the characteristics that define a fertile sperm cell. It is known that viable sperm are capable of swimming in a straight-forward direction (progressive motility), and have an intact sack of enzymes (acrosome) surrounding the top of the head, utilize nutrients from dilutors and secrete waste products (metabolize) that may alter the pH of the dilutor, and have a characteristic normal shape (morphology).

CONCENTRATION:

The accurate measurement of semen is important when semen is to be used in artificial insemination or processed for freezing. The concentration of buck sperm reported to be necessary for a satisfactory fertilization rate is 40 million sperm per ml when fresh semen is used and 120 million motile sperm when frozen semen is used (Corteel, 1975). However, personal experience has indicated that much fewer cells are required if semen is of good quality and AI procedures are proper. Semen put up at a concentration of 120 million or 60 million per 0.5 cc straw and having a 50% post-thaw survival rate yielded suitable fertilization. This is of course subject to animal variation and I would suggest using the higher concentrations unless semen from individuals are field tested for fertility versus concentration.

The concentration of sperm in a sample can be determined by the use of a hemocytometer (available from almost any distributor of laboratory supplies), or by photoelectric colorimetry.

The concentration of the ejaculate is a function of several parameters. They include the degree of sexual preparation of the buck, the age of the buck, the time of year the collection is made, the amount of sexual rest before collection, the health of the buck, his nutritional state, inherent sperm storage, and the production capacity of the buck.

Most of the factors can be controlled by employing good management practices. The ability of the buck to produce sperm (in the testes) and store sperm (in the epididymis) can be assessed to some extent by palpation and measurement of the testes and epididymis. In bulls, these measurements serve as a good predictor of sperm output, and it is reasonable to assume the same is true for the buck.

Since it is well known that there is a direct relationship between the number of sperm inseminated and fertility, it is important that concentration estimates are determined accurately. An over-estimate may result in over-dilution of the sperm and consequently reduced conception rates.

As mentioned before, the total sperm harvest is determined by multiplying the ejaculate volume times the concentration. It is the total number of sperm collected that is the important semen quality parameter. The average buck ejaculate usually contains between 2 and 9 billion sperm cells. Between March and August, at Northern latitudes, the volume of ejaculate is usually low (0.4 to 0.6 ml), which concentration is relatively high (5 to 9 billion sperm/ml). During the breeding season, the reverse is usually true; volume may range between 0.8 and 2.0 ml and concentration may range from 2 to 6 billion sperm/ml).

VOLUME

The volume of the ejaculate will generally be between 0.5 and 1.5 ml. Blokhuis (1962) estimated the average volume from a series of collections to range between 0.25 and 5.0 ml with ideal volume not to exceed 2 ml. Smith (1978) reported the average buck semen volume to be between 0.5 and 1.0 ml; however, averages of 0.85, 0.82, and 0.68 ml have been reported by Huat (1975), Patel (1967), and Eaton (1952), respectively. Volume is an important criterion in semen evaluation. The quality of the semen may decrease as the total volume of the ejaculate increases (Huat, 1976). But, generally larger volumes mean more sperm. Older bucks generally have a larger volume of ejaculate than younger bucks. Therefore, age should be considered with respect to this parameter of semen evaluation. The volume is simply measured using the graduations on the collection tube.

MOTILITY

The progressive motility of sample is defined as that percentage of the spermatozoa in a sample that swim in a more or less straight-forward direction. Circular or reverse motion often indicate cold shock or media that is not isosmotic with semen. The progressive motility is determined by examining a drop of semen, diluted so that individual cells can be visualized.

Physiological saline can be used as a diluter, but it is better to use a buffered solution containing an energy source such as glucose. Phosphate buffered saline (PBS) with 1% glucose works very well. Other standard buffered solutions (such as Ringers solution, sodium citrate buffer, Tris buffer, and Tyrodes solution) can also be used.

It is extremely important that the solutions, pipettes, and glassware (such as test tubes and slides) be at the same temperature as the semen. It is best to make the motility estimate as

soon after the semen is collected as possible. Since good semen handling dictates that the collection tube should be at 37°C at the time of collection, all of the things that come in contact with the semen should be at 37°C. Remember, semen is and never will be any better than the instant it is ejaculated. One item that is often neglected is the temperature of the microscope stage. Although the semen does not come in direct contact with the stage, within a few seconds of placing the slide on the microscope, the slide temperature will be that of the microscope. To avoid this problem, a device known as a stage warmer can be used to keep the slide at 37°C while it is being examined.

Since the motility measurement is subjective (one actually makes an "educated guess" of the percentage of motile sperm), care should be taken to make the sample as "readable" as possible. Ideally, the sample, under the cover slip, should be as thin as possible (one sperm thick) and each field should contain between 10 and 20 sperm. On the average, this can be achieved by mixing 6 to 8 microliters (ul) of semen with 0.4 ml of diluter and transferring 7 ul of that mixture to the slide. **A less desirable but more practical approach is to place a drop of diluter, about ½ the size of a dime, on a pre-warmed slide and transfer a small amount of semen to the drop with a solid clean glass rod**. The rod should be about the diameter of an AI straw or smaller. Mix and then transfer part of the mixture to a clean part of the slide and carefully put the cover slip in place before examining. My personal preference is to evaluate semen in its final extended concentration so that I have a direct comparison with what I see when a straw is evaluated after thawing.

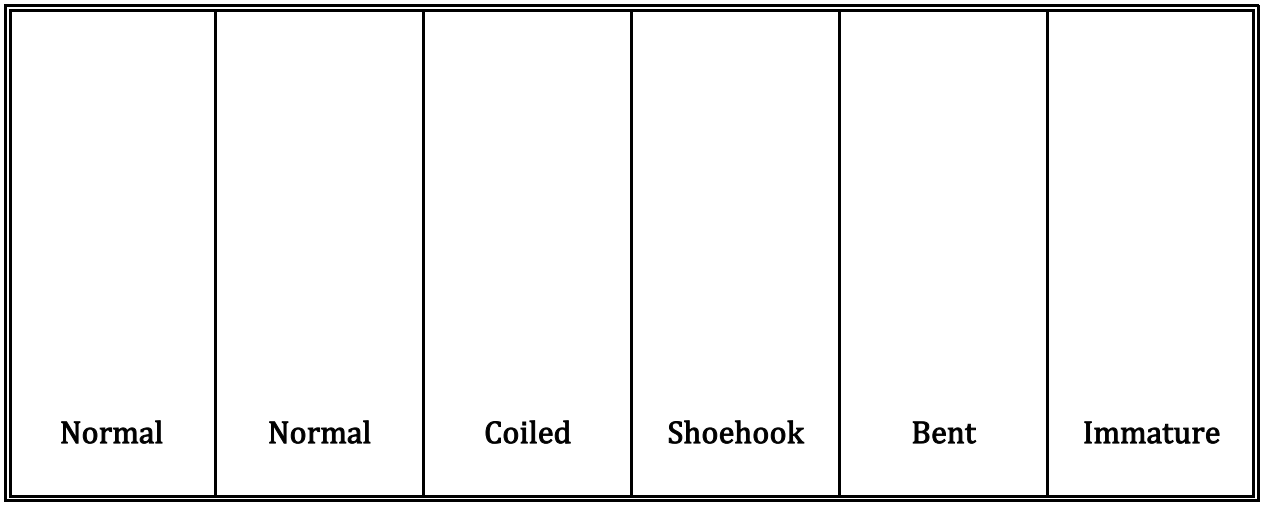
Sperm are evaluated in a number of different areas, using 2 or 3 preparations of the same sample. Sperm cells near the edge of the coverslip or near trapped air bubbles do not

appear to act "normally". Most reports indicate (Huat, 1975; Smith, 1978) an average motility score of 85%. Semen with an initial motility much below this should probably not be processed any further.

MORPHOLOGY

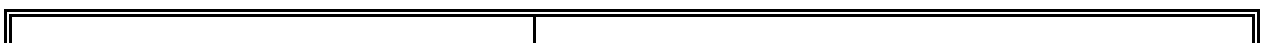
When sperm morphology is evaluated (Fig. 5), one usually looks for abnormal shapes of the head and tail. It has become popular to examine the morphology of a portion of the head, the acrosome. Examination of the acrosome requires the use of a fairly sophisticated microscope fitted with either phase-contrast or differential interference optics. Even though researchers find measurement of acrosomal integrity helpful, most bull studs to do not perform this evaluation because of the time and expense in making this measurement.

FIGURE 5: Some Traits of Normal and Abnormal Semen.



See following pages for additional morphological traits.

FIGURE 5A:



Normal Spermatozoan and Loose Cytoplasmic Droplets	
HEAD ABNORMALITIES	
A. Large (Giant, Macrocephalic)	
B. Small (Dwarf, Microcephalic)	
C. Double	
D. Underdeveloped (Unripe)	
E. Pyriform	
F. Round	
G. Narrow (Elongated)	

FIGURE 5B:

ACROSOME ABNORMALITIES	
A. Raised Knobbed	
B. Pointed Knobbed	
C. Beaded Knobbed	
D. Flattened Knobbed	
E. Ruffled	
F. Folded	
G. Unilaterally Incomplete	
H. Bilaterally Incomplete	
I. Swollen	

FIGURE 5C:

MIDPIECE ABNORMALITIES	
A. Thick	
B. Knob	
C. Filiform	
D. "Moth Eatten"	
E. Abaxial	
F. Kink or corkscrew	
G. Disassociated	
H. Double	
I. Split	
J. Defective Attachment	
K. Offshoot Defect	
L. Broken	
M. Coiled Around Head	

FIGURE 5D:

TAILPIECE ABNORMALITIES	
A. Coiled 1. Tail Only 2. Tail and Midpiece a. Loose b. Tight	 a. b.
B. Bent 1. Single or Simple	

2. Double	
C. Cytoplasmic Droplets 1. Proximal 2. Distal	

FIGURE 5E:

OTHER CELLS OCCURRING IN SEMEN	
A. Medusa Formation	
B. Epithelial Cells	
C. Leukocytes	
D. Erythrocytes	
E. Germinal Epithelium "Spheroids"	

Sperm cellular abnormalities are categorized as primary and secondary abnormalities. Primary abnormalities are those that have occurred during spermatogenesis. Secondary abnormalities are those that have occurred during the later stages of development, during ejaculation, or during the collection process (Herrick and Self, 1962). Primary abnormalities are reported by Herrick (1962) and include all abnormalities of the head, coiled tails, double forms (two heads, midpieces, or tails, abaxial midpieces (midpieces that attach to the head off-center), medusa formations (ciliated globular masses), abortive tails and spheroids (round headed sperm). Secondary abnormalities include loose heads, droplets of cytoplasm on the tail or midpiece, bent tails, detached heads, and bodies in the sample other than spermatozoa (red blood cells, white blood cells, bacteria, squamous epithelial cells or spermatozoal clumps). Usually, the percentage of morphological abnormalities in the semen of a buck with normal fertility should be less than 5% during the breeding season. If collections are made during the summer, the percentage of abnormalities can be expected to be higher. The percentage of morphological abnormalities in the semen of below average and poorly fertile bucks may be 10-15%, and 15%+, respectively (Easton and Simmons, 1952; Huat, 1973; Ott, 1978; and Patel, 1964).

Sperm can be stained with a supravital ("live-dead") stain. This helps in the visualization of morphological abnormalities and also can be used as a measure of the integrity of the cellular membrane surrounding the sperm cell. A drop of diluted semen is placed on a warmed microscope slide (37°C) and mixed with a drop of stain, such as eosin-nigrosin. Then a thin smear of the mixture is drawn across the slide, and the slide is dried. A coverslip is applied

to the preparation and sample is examined at 100-500X with an oil immersion objective. The sperm that were alive at the time the slide was prepared will not stain and will look white or clear. The dead cells will stain pink.

COLOR AND CONSISTENCY

The color of buck semen ranges from a creamy white color to yellow. These ranges of color have been reported by a number of authors and apparently do not have any bearing on the semen quality. Off colors, however, should be noted and whenever possible should be cultured. Red coloring of the sample indicates the presence of blood. This may be due to adhesions or trauma that has occurred prior to collection. Yellow watery colors may indicate urine in the ejaculate. A black or dark sample may indicate debris such as dirt or manure contaminating the sample due to an uncleaned prepuce or the presence of pus.

pH

The pH of the semen is sometimes measured at the time of semen collection. This determination is usually made by using litmus paper or a pH meter. The average pH range of a semen sample obtained from a buck is between 6.5 and 7.4. Huat (1975) estimated the average pH range to be between 6.5 and 7.7 and Patel (1967) reported an average pH to be 6.5. The pH of the semen sample may be different during different times of the year. According to Williams (1981), if the semen sample is too basic, citric acid can be added to adjust the pH and sodium bicarbonate added to acidic samples will bring the sample to the desired pH. Anything that alters the pH of the semen above or below the normal range will cause sperm death. The presence of blood or infection can alter the pH enough to kill the sperm.

If the pH of the ejaculate is low (acidic) that may not indicate poor quality since highly

active sperm samples produce lactic acid as a metabolic waste product. However, the sperm will be killed if the acidity is not neutralized. All of the common semen extender (dilutors that "extend" sperm cell life) are buffered. That is, they contain chemicals that tend to prevent pH changes and "force" the pH to remain in a safe range. Since it is a good practice to dilute buck semen as quickly after collection as possible, to prevent loss of viability, the extender will usually correct any pH problem.

CONCLUSION

The areas of semen collection, processing, extension, freezing, and evaluation are each individually extensive and vital to the success of an AI industry. Each of these areas are still evolving and hopefully improving. In the final analysis, the proof of progress lies in the effectiveness of the results. Although progress in these areas with reference to its application in the caprine species has not progressed as fast and as far as with cattle, the need is still there. With the major populations of goats residing in the under-developed countries, there is the obvious demand to accelerate our understanding in the fields with the goal to boost the productivity for these countries. Combining well-designed research programs whose objectives are to provide answers for reputable AI organizations, semen processors, etc., is and should be the ultimate goal.

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