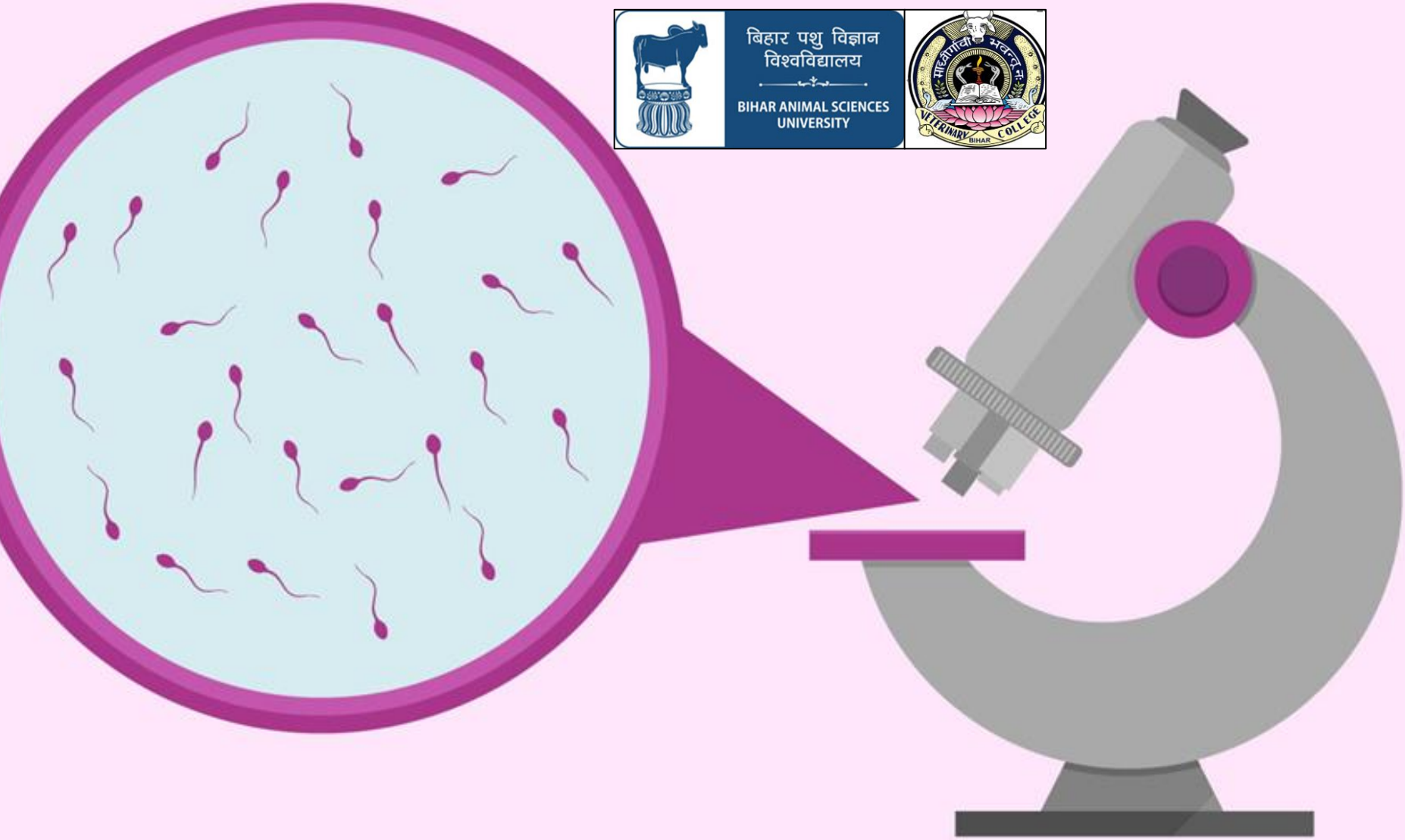
Factor Affecting Semen Quality, Semen Culture, Test for Assessment Sperm Motility, Sperm Survival and Fertilizing Capacity



UNIT 5 VGO 604

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Introduction

- Semen quality, like any other phenotypic expression
- The genetic component is generally thought to be influence the heritability of fertility usually low
- Fertility varies substantially among bulls
- Bull testes must be 2 to 6°C cooler than core body temperature for fertile sperm
- Compensable sperm abnormalities can be overcome by increasing the dose used for artificial insemination

Factors affecting semen quality

- Age
- Seasons
- Temperature
- Nutrition
- Diseases
- Frequency of Semen collection
- Management at the time of semen collection
- Pollution

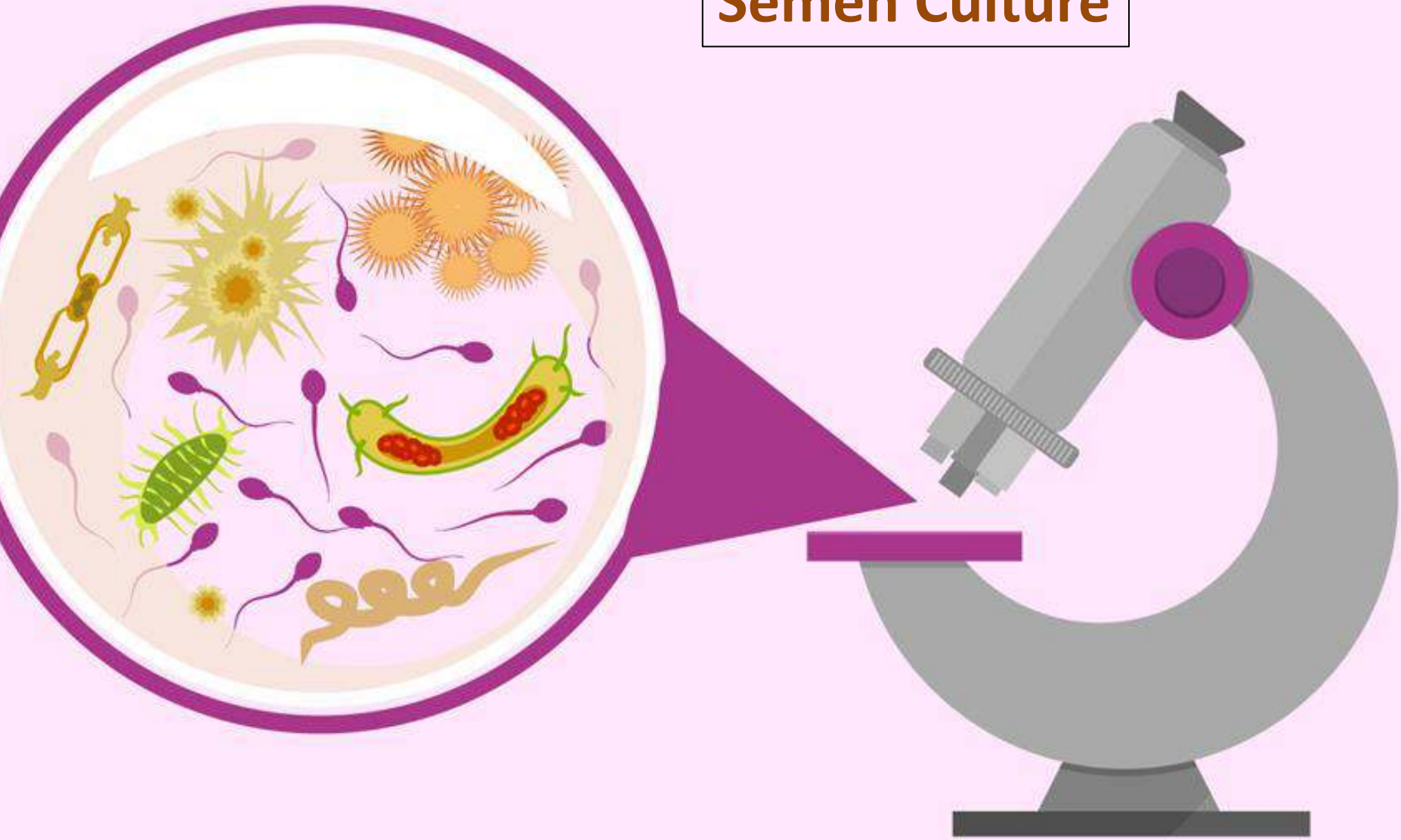
Routine Examination

- ❑ Volume
- ❑ Colour
- ❑ Consistency
- ❑ Mass motility
- ❑ Individual motility
- ❑ Concentration

Periodical Examination

- ❑ Estimation of Live and Dead
- ❑ Estimation of percent sperm abnormality
- ❑ Resistance tests
  - Incubation tests
- ❑ Metabolic Activity test
  - MBR test
  - Fructolytic index tests
- ❑ Catalase test
- ❑ Functional Assessment test
  - CMPT
  - Penetration of Zona free hamster test
  - HOST

Semen Culture



Standard Plate count (SPC)

- The samples processed for standard plate count will collected under strict aseptic condition.
- Fresh semen samples processed for bacterial counting will used within half an hour of collection while post-thaw semen samples will used after 24hr of freezing.
- Standard plate count will be done by spread plate technique in duplicate plates as per Cruickshank *et al*., 1975.
- Tenfold serial dilution from 1:10 to 1:1000 will made for all preputial washing samples for standardization of SPC. Subsequently 1:10 to 1:100 dilutions will be used for bacterial count from preputial washing samples and 1:10 dilution will be used for bacterial count from fresh and post-thaw semen samples.
- The normal physiological saline solution will be used as routine diluents for all the samples.
- The inoculated nutrient agar plates will be allowed to dry for 10 minute before incubation at 37^0C for 24 h. The bacterial count (cfu/ml) of each sample will be counted by multiplying the dilution factor with number of colony in plate.

Average fertile life of sperm and ovum

Species	Sperm (Hrs.)	Ovum(Hrs.)
Cattle	30-48	20-24
Horse	72-120	6-8
Human	28-48	6-24
Rabbit	30-36	6-8
Sheep	30-48	16-24
Swine	24-72	8-10

Table 2 The effect of temperature on survival of bovine sperm at 37°C after storage for 6–128 h. The end point was the time at which 10% of the sperm were still motile. (n= 10; Trial 2)

Temperature of storage (°C)	Period of storage (h) 6	24	48	120	168	Average
15.6	61.9	59.6	57.0	58.8	53.7	58.2
21.1	62.5	60.0	57.4	59.3	54.9	58.8
26.7	60.7	55.5	54.5	53.3	45.3	53.9
32.2	59.9	48.6	42.6	36.6	24.8	42.5
Average	61.3	55.9	52.9	52.0	44.7	53.4

Survival of buffalo bull spermatozoa during freeze-thawing and post-thaw incubation can be enhanced more with (ALA alpha-lipoic acid) than CLC or control, followed by CLC (cholesterol-loaded cyclodextrin) than control.

Most sperm die within minutes after ejaculation inside the vagina or outside the woman's genital tract. However, once sperm enters the woman's genital tract, cervix, and uterus, some can survive up to 5 days, though most will not survive longer than 1-2 days.

What determines how long sperm live?

Sperm can live from several minutes to several days depending on their environment:

- Sperm exposed to air, deposited on clothing, bed linens, or toilet seats, for example, dry out very quickly and die, usually within minutes after ejaculation

- A sperm sample collected in a sterile container at body temperature may remain alive for several hours, but the sperms' fertilizing capability drops dramatically within 60 minutes after ejaculation.

- Sperm that moves from the vagina into the uterus can survive longer. Studies have shown that conception is possible up to five to six days after intercourse. It is quite possible for some sperm to survive that long in the uterus, usually in the cervical mucus or the fallopian tubes. However, that is not typical. Most sperm die in the uterus within 24-48 hours after ejaculation, and the more time has passed after ejaculation, the less likely is the fertilization of the egg.

- In excellent laboratory conditions and in a nutrient medium, they can remain alive for up to seven days.

- Sperm can be frozen at extremely low temperatures (but not in the refrigerator) and survive for years
- Sperm can survive in the vagina for up to several hours.

Fertilizing capacity of spermatozoa

In most mammals, natural insemination of the female occurs only during estrus. The interval from the onset of estrus to ovulation may be several hours or even a few days [1] and sperm must retain their fertilizing capacity for this period. In contrast, the fertile lifespan of oocytes is generally less than half that of sperm [1]. Thus, there must be mechanisms to extend the fertile lifespan of sperm in the female reproductive tract. There is evidence that the caudal oviductal isthmus functions as a sperm storage reservoir and that sperm may be held in the reservoir by binding to the mucosal epithelium [2–7]. In cattle, approximately 8 h may be required after insemination for sufficient sperm to accumulate in the isthmic reservoir in order to ensure a high fertilization rate [8]. Since the period from onset of estrus to completion of ovulation may be as long as 30 h in cattle, sperm destined to fertilize may spend up to 22 h in the isthmus [5]. Therefore, some of the mechanisms of maintaining sperm fertilizing capacity may exist in the isthmus.

Two recent technical advances have made it possible to examine in vitro the mechanisms involved in sperm storage in the bovine oviduct. The first of these is the ability to maintain organ-specific function in cells cultured in vitro [9], including endosalpingeal epithelial cells [10]. The maintenance of a polarized and differentiated cell population entails establishing primary cultures on components of basal laminae that have been layered onto elevated permeable membranes [11, 12]. The second advance has been the development of highly successful and repeatable in vitro maturation and fertilization techniques for bovine oocytes [13]. In this study, these techniques were used to create an in vitro system that enabled us to investigate whether the motility and fertilizing capacity of bovine sperm could be prolonged by oviductal epithelium.