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# Sexed Semen in Buffalo

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Sexed semen technology has evolved from early philosophical concepts to contemporary flow cytometry that can distinguish between X- and Y-bearing sperm.By facilitating tailored offspring sex, helping dairy systems that need more female calves for replacement and milk productivity, and supporting meat systems that gain from faster-growing males, it offers significant benefits in buffalo production. Estrus-synchronized cows inseminated with sexed semen generated using methods like the albumin gradient method are used in standard evaluation. However, because to cryopreservation damage, decreased sperm functioning, and embryo loss, sex-sorted semen continues to have lower reproductive outcomes. By strengthening sperm integrity and removing aberrant cells, nanoparticles may improve the quality of sexed semen, according to recent research, providing a viable remedy for present problems.

## Introduction

Every new life starts with a spermatozoon, which is a single microscopic cell. This tiny performer moves through semen, a complicated fluid composed of secretions from the male reproductive tube and auxiliary glands as well as millions of sperm cells. When talking about contemporary reproductive tools like sexed semen, these terms are frequently used interchangeably even though they refer to distinct physiological entities. Since Hippocrates and the early Greek philosophers centuries ago, people have been interested in affecting the sex of their offspring. Because he thought that "generative heat" had an impact on sex, Aristotle even recommended that a woman looking for a male child lie on her right side. Antonie van Leeuwenhoek's 1677 discovery of spermatozoa and Lazzaro Spallanzani's early artificial insemination studies in frogs (1777) and dogs (1780) were significant advances in scientific knowledge. But the real scientific basis of sexed semen technology didn't start to take shape until the 1970s, when scientists thoroughly examined the structure of sperm, including its DNA content, chromosomal variations, and physical characteristics. This led to the creation of flow cytometry, the most accurate technique for distinguishing between X- and Y-bearing sperm. Sexed semen is now a potent tool for improving genetics, directing herd composition, and increasing reproductive efficiency in a variety of animals. Because of their distinct physiology, buffalo are developing this technology more slowly than

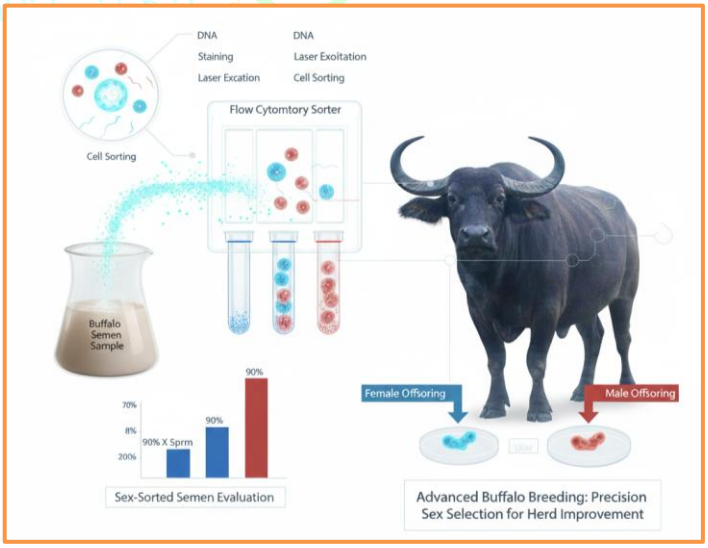


Figure 01: Illustration showing buffalo with flow cytometry technique.

cattle, who embraced it earlier(Kumar *et al.,* 2025).Now, with accumulating research and successful field applications, buffalo reproduction is entering a phase where the age-old desire to predetermine the sex of offspring is finally supported by reliable, evidence-based science.

## Drivers for Adopting Sexed Semen Technology

In buffalo production systems, the key breeding aims are to induce lactation, ensure a consistent supply of replacement females, and support meat output, all of which rely on successful conception. In dairy buffalo herds, artificial insemination with frozen-thawed semen has grown in popularity, especially in Southeast Asia and India, where genetic improvement initiatives mostly depend on AI. Similar to cattle, buffalo's sex ratio may be modestly impacted by the use of AI and frozen semen, while different studies have found different outcomes (Perera, 2011). Producing a targeted sex distribution among offspring is the primary reason buffalo adopt sex-sorted semen. Female calves are particularly important in dairy buffalo systems because they influence long-term milk production, replacement stock, and future herd size. Buffalo heifer calves are continually in high demand due to the species' slower reproductive turnover and the economic importance of milk from river buffalo breeds such as Murrah, Nili-Ravi, and Jaffarabadi (Singh and Balhara 2016). Additionally, research suggests that buffaloes with female fetuses may have better lactation performance in the next cycle, albeit this finding needs to be confirmed under various management scenarios (Borghese and Moioli, 2016). Male calves are favored in meat-focused buffalo systems, especially swamp buffalo in Southeast Asia, due to their superior growth rates and carcass features (Wanapat and Chanthakhoun, 2015). Therefore, buffalo sex-sorted semen aligns offspring sex with system-specific economic goals, providing strategic advantages for both dairy and meat production.

## Methodology

Clinically healthy adult buffalo cows between the ages of five and nine who are kept under conventional management circumstances should be used for the study. The selection of animals should be based on their normal physical state and lack of reproductive abnormalities. A two-dose prostaglandin (PGF) strategy should be used for estrus synchronization. Five milliliters of PGF should be injected intramuscularly into each cow, and then again eleven days later. This synchronization regimen would then be used to plan fixed-time artificial insemination (FTAI). Mature, healthy breeding bulls should provide their semen. Spermatozoa should be sexed using the albumin gradient technique, which involves dissolving egg white (albumin) in Tris buffer according to standard laboratory protocols. Sexed semen straws would be prepared and stored under standard cryopreservation conditions. Artificial insemination should be performed 72 hours after the second PGF injection. Each buffalo cow would receive two straws of sexed semen via the FTAI method. Following insemination, cows should be administered 2 ml of hCG intramuscularly. To avoid natural mating, inseminated cows should be separated from bulls for 2–3 weeks.

## Limitation associated to sex semen

Fertility results using sex-sorted sperm in ruminants remain below expectations, regardless of the insemination method utilized. Although the optimal sperm dose, synchronization, and AI procedures are necessary for successful outcomes, frozen-thawed sexed semen frequently exhibits low rates of conception, lambing, and calving.(Magopa *et al* 2023) Cryopreservation-related damage, which modifies sperm proteins, lipids, and ions and degrades sperm quality after thawing, is mostly responsible for this decreased fertility. Because bovine sperm are more resilient to freezing, cattle do relatively better(Bayril, *et al.*,2023). There have also been reports of higher pregnancy and embryo losses, which are probably caused by degraded embryo quality, improper AI timing, or reduced sperm function after sorting and multiple freezing processes. Similar developmental losses happen in vitro as a result of culture media components, oocyte treatment, and semen quality.

## Application to improve sexed semen

Nanoparticles, ultra-small, highly customizable structures with a vast surface area, are rapidly being investigated in reproductive health due to their versatility and ability to be manufactured from materials like as metals, proteins, and polysaccharides (84). Their use in assisted reproductive technologies has grown quickly, enabling developments in follicle culture, IVF, artificial insemination, in vitro embryo formation, and, most significantly, semen sex-sorting (DeGraaf *et al.*, 2007). Pre-gender selection reduces genetic waste, produces the desired sex, and speeds up genetic advancement in breeding programs, among other advantages (Dominguez *et al*., 2018). However, despite its promise, sex-sorted semen often results in lower conception rates, largely due to irreversible sperm damage caused during the sorting process (59). This challenge has driven efforts to enhance the quality of sexed semen across fresh, chilled and frozen-thawed forms (Bathgate *et al.*, 2013). Increasing evidence across species indicates that nanoparticles can improve semen quality and fertility outcomes (Seidel,2011) in pigs, for instance, nanoselection has successfully eliminated abnormal spermatozoa, leading to improved reproductive performance.

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