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Case Study on the Use of Assisted Reproductive Techniques in Improving Water Buffalo Fertility

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Abstract Water buffaloes play a significant role in livestock production in many regions of Asia, undertaking multiple functions such as dairy production, meat processing, and draft use. However, its reproductive efficiency is relatively low, such as long postnatal intervals and difficulty in identifying estrus, which have long restricted the improvement of production performance and the progress of germplasm improvement. This study systematically reviewed the reproductive biological characteristics of water buffaloes, the limitations of natural reproduction, and analyzed the mechanism of ARTs in improving reproductive performance, including enhancing conception rates, synchronous estrus, and accelerating genetic progression. Through the case analysis of the Indian Buffalo Breeding Center, the artificial insemination promotion project in the Philippines, and the OPU-ET experimental platform in southern China, this study evaluated the effectiveness, advantages, and technical and management challenges faced by ARTs in practical applications. The research results show that although ARTs can significantly improve the reproductive efficiency of water buffaloes and promote the rapid spread of superior genes, its large-scale promotion still relies on cost reduction, farmer training and the construction of a good supporting system. This study aims to reveal the mechanisms by which these techniques improve the reproductive performance of water buffaloes, shorten their reproductive cycles, and promote the expansion of superior populations, and to provide theoretical support and practical references for establishing a scalable and sustainable water buffalo breeding technology system.
Keywords Water buffalo; Assisted reproductive technology; Reproductive capacity; Artificial insemination; Embryo transfer

1 Introduction

The buffalo (*Bubalus bubalis*) is not a species that has only started to attract attention in recent years. Long ago, it had already been a "jack of all trades" in the hands of farmers in parts of Asia, South America and even Europe - capable of producing milk and meat as well as working in the fields. This kind of animal has a strong adaptability to the environment and is not bad at resisting diseases. It is particularly suitable for two extreme production systems: small-scale family breeding and large-scale livestock farms. Moreover, in terms of nutritional components, its milk fat content and protein content are generally much higher than those of cattle, which is indeed very attractive to the dairy industry (Coman et al., 2024).

But then again, the advantages of water buffaloes cannot cover up their shortcomings in reproduction. Compared with cattle, it is indeed a bit of a "backer" in terms of reproduction. For instance, sexual maturity comes slowly and estrus is not obvious. Sometimes a female water buffalo does not "speak" for several months, and the breeders cannot catch the right time to mate. In addition, due to strong seasonality, long calving intervals and slow postpartum recovery, the reproductive efficiency is naturally not high. These problems are also vulnerable to the influence of the environment, nutrition, diseases and breeding methods. Once there are problems, the conception rate drops immediately and the breeding benefits also shrink accordingly (Devkota et al., 2022). Some studies have pointed out that this kind of low fertility not only slows down the population renewal rate, but also affects the sustainable development and economic returns of the entire buffaloe-based livestock system (Nava-Trujillo et al., 2020).

To solve this long-standing and difficult problem, it is impossible to just "wait for it to get better on its own". Assisted reproductive technology (ARTs) thus emerged, including artificial insemination (AI), timed artificial insemination (FTAI), in vitro embryo transfer (IVEP), and embryo transfer, among others. They do not simply increase the number of mating times. Instead, they bypass the natural reproductive problems of water buffaloes through means such as synchronous ovulation, concentrated estrus, and shortened intervals - especially during periods when estrus is not obvious or the season is incorrect, they can also improve reproductive efficiency (Srirattana et al., 2022; Maylem et al., 2025). However, these technologies are not simply "put on". They must be optimized around the physiological characteristics of water buffaloes themselves and managed more meticulously in order to truly reduce costs and improve effects (Baruselli et al., 2018).

This study aims to emphasize the economic and genetic value of water buffaloes in global animal husbandry, analyze the main reasons and impacts of the species' low reproductive capacity, showcase the application and case studies of assisted reproductive technology in enhancing reproductive capacity, and is expected to provide information for breeding strategies, increase productivity, and support sustainable water buffalo farming.

2 Biological Basis of Reproduction in Water Buffalo

2.1 Characteristics of the reproductive cycle and estrus behavior in buffalo

Although the average estrus cycle of water buffaloes is 21 days, it is actually not stable and often fluctuates. The most troublesome thing is that their estrus is not very obvious, unlike that of cattle which is easy to notice. Sometimes it only lasts for five or six hours, and sometimes it can extend to a whole day (5 to 27 hours), which makes it very difficult for breeders to accurately grasp the timing for mating. Moreover, unlike cows, water buffaloes do not have obvious homosexual climbing behavior. Without even this "hint", it is even more difficult to detect estrus. Ovulation usually occurs between 24 and 48 hours after the onset of estrus. However, in poor conditions, water buffaloes are prone to delayed sexual maturity, slow postpartum recovery, and prolonged estrus periods (Nava-Trujillo et al., 2020; Kolachi et al., 2025), which is also quite a headache.

2.2 Constraints on reproductive performance

There are actually many reasons that affect the reproductive efficiency of water buffaloes, and they are often intertwined. The first is seasonality. Water buffaloes prefer to breed under short-day conditions, so their breeding performance is even better in autumn and winter. However, as the duration of sunlight increases in spring and summer, their estrus actually decreases and their ovulation also drops. This is because light exposure affects the secretion of melatonin, which in turn regulates the entire hypothalamic-pituitary-ovarian axis (Ramadan, 2017; Currin et al., 2022). Another problem is the corpus luteum. Sometimes it is embedded in the ovary and is not easily observed. Coupled with weak luteal function and low progesterone secretion, the estrous cycle and pregnancy rate will be affected (Devkota et al., 2022). Nutrition should not be overlooked either. Malnutrition, extremely hot weather or parasitic infections can all cause water buffaloes to enter a "low desire" mode and not cooperate with breeding arrangements, especially during the non-breeding season or when feed is tight (Zicarelli, 2019). There is another phenomenon called "silent estrus" or "ovaries without a cycle". Many water buffaloes just quietly "miss" the estrus period, and the breeders are completely unaware of it. Naturally, they cannot be mated and the conception rate is also low.

2.3 Physiological differences in sperm and oocyte traits

Buffaloes also have some special features in terms of gametes. Let's start with the female. The ovaries of water buffaloes are inherently smaller than those of cows, and they contain fewer primordial follicles, which means that the "raw materials" available for the development of oocytes are limited. Moreover, their follicular development usually follows only one main path, with one dominant follicle, and the others are prone to premature atresia. As follicles grow, the gene expression of oocytes and granulosa cells within them also changes. This difference directly affects the continued development of follicles and the quality of oocytes (Xu et al., 2024). Let's take a look at male water buffaloes. The performance of their sperm is actually related to the season. The quality of sperm is generally better in winter - including indicators such as motility, membrane integrity, and fallopian tube binding ability. In contrast, it is poorer in summer. Some studies have also pointed out that some genes related to gonadotropin release, such as SNP polymorphisms in GnRH, can affect sperm motility, concentration and morphology (Saraf et al., 2019; Wang et al., 2020). Generally, the offspring of bulls with good integrity of the sperm membrane and acrosome are also more likely to successfully conceive. These biological characteristics of water buffaloes themselves - whether it is the estrous cycle, ovarian structure, or gamete function - all indicate one thing: reproductive management cannot be simply copied from the water buffaloes. Strategies must be adjusted according to the particularity of water buffaloes, and at the same time, various assisted reproductive technologies must truly "adapt" to their physiological rhythms in order to increase the success rate.

3 Overview of Assisted Reproductive Techniques (ARTs)

3.1 Development and limitations of Artificial insemination (AI)

Although artificial insemination (AI) has been used in the field of buffalo breeding for many years, the results have never been satisfactory. Compared with cattle, the water buffalo's less "cooperative" estrus behavior has long been an old problem - sometimes the cycle is unstable, sometimes the season is particularly affected, and the estrus is not obvious. These factors make estrus detection quite a headache and it's also difficult to grasp the timing of mating. To bypass this problem, the timed artificial insemination (FTAI) program was later introduced. This approach does not require observing estrus and controls ovulation time through medication, which can slightly increase the success rate of mating and is also convenient for centralized management (Lui, 2025). But then again, even so, the conception rate has not been able to increase steadily, especially during the non-breeding season. Factors such as heat stress, the operator's experience level, semen quality, and even the reproductive state of the cow at that time can all affect the final outcome. Some studies have also pointed out that even with concurrent estrus treatment, the overall success rate of water buffaloes is still lower than that of cattle (Atabay et al., 2020; Neglia et al., 2020; Atabay et al., 2024; Kolachi et al., 2025; Maylem et al., 2025).

3.2 In vitro embryo production (IVF) and embryo transfer (ET) techniques

The entire set of IVF and ET technologies developed from cattle is now also being attempted to be applied to water buffaloes. However, the problem is that the number of follicles in the ovaries of water buffaloes is relatively small, and the maturity of oocytes is often insufficient, which makes the entire in vitro embryo production (IVEP) process more complicated than that of water buffaloes. The entire process roughly includes the collection, maturation, fertilization and culture of oocytes, and finally the embryo is transplanted into the recipient cow that is already "ready". Although it sounds like a standardized operation, different donors, breeding bulls, and even environmental conditions can all affect the embryo development rate and yield. Even so, many studies have confirmed that normal pregnancy and even successful calving can be achieved whether using fresh embryos or cryopreserved vitrized embryos. This also indicates that these techniques do have their potential in genetic improvement and protection (Baruselli et al., 2023; Bandeo et al., 2025).

3.3 Oocyte pickup and in vitro maturation (OPU-IVM)

Nowadays, more and more breeding projects are beginning to pay attention to OPU, especially the use of ultrasounds guided transvaginal oocyte retrieval (OPU) or laparoscopic methods (LOPU) to repeatedly retrieve eggs from live cows or even pre-estrus heifers. One of the benefits of doing so is that it can accelerate the replacement of generations and shorten the breeding cycle. Although the OPU-IVM technology of water buffaloes developed more slowly than that of cattle, in recent years, by adjusting the hormone stimulation methods, such as using FSH or eCG, there has been an improvement in both the quantity and quality of eggs. However, at present, the number of available embryos that can be retrieved from each OPU is still less than that from cattle, and the differences in ovarian responses among individuals are also quite obvious. Therefore, the subsequent focus may still have to be on how to optimize the cultivation conditions and stimulation schemes. Only in this way can the OPU-IVM technology truly play a stable and long-term role in the buffalo breeding program (Currin et al., 2022; 2023).

4 Mechanisms of ARTs in Enhancing Buffalo Fertility

4.1 Physiological mechanisms for increasing conception rate and shortening estrus cycle

In terms of breeding management, the problem with water buffaloes has never been merely "incompatibility", but more often it is "inability to develop passion". The estrus is not obvious and the ovulation time is unstable. Under such circumstances, relying on manual observation of estrus for mating is not very reliable. As a result, methods like timed artificial insemination (FTAI) have gradually gained attention. Its core lies in using hormone intervention to bring ovulation time within a "controllable range". Drugs such as GnRH, hCG, prostaglandins and progesterone can all be used. The key is to combine them reasonably and smooth out the rhythm of the follicular phase and the luteal phase. Whether estrus can be detected or not is not that important. Compared with natural estrus or fertilization arranged by experience, the pregnancy rate of this method is significantly more stable (Presicce et al., 2022; Bhat & Dhaliwal, 2023). Sometimes, adding an injection of GnRH or hCG during artificial insemination can further increase the success rate. Moreover, this approach is not expensive in field practice and is easy to promote in operation (Atabay et al., 2020; Selvaraju et al., 2023).

4.2 Herd management through estrus synchronization and superovulation

If you only want one or two cows to get pregnant smoothly, using FTAI alone might be sufficient. But when it comes to an entire group of water buffaloes, especially considering management costs and labor constraints, simultaneous estrus becomes even more cost-effective. Synchronization solutions like Ovsynch and CIDR have actually been used quite proficiently by many farms. They can unify the estrous cycles of multiple water buffaloes, eliminating the need to observe each one individually during mating and saving a significant amount of time. As for superovulation, it is more like a tailor-made tool for IVF or embryo transfer. By injecting hormones such as FSH and eCG, cows can release multiple mature follicles at once, thereby increasing the efficiency of egg retrieval. If it is a donor with high genetic value, this approach is obviously more cost-effective and can amplify the "leverage effect" of genetic output more effectively, which is quite practical both in the breeding season and the non-breeding season (Baruselli et al., 2018; 2020).

4.3 Sperm quality control and accelerated genetic progress

Ultimately, whether it is artificial insemination or in vitro fertilization, sperm quality is the foundation. Although many semen samples may remain intact when frozen, once the sperm motility and membrane integrity do not meet the standards, mating will be in vain. Nowadays, some technologies can be of assistance, such as proteomic analysis and genetic screening - markers like *GnRHR* gene polymorphisms have been used to assess sperm expressiveness (Figure 1). This way, bulls with potential infertility or low breeding value can be screened out before mating, saving wasted time (Wang et al., 2017; Karanwal et al., 2023; Kolachi et al., 2025). From a broad perspective, these technologies not only increase the conception rate, but more importantly, they promote the rapid spread of superior genes within the population, accelerate the speed of genetic improvement of water buffaloes, and also provide a practical handle for enhancing breeding efficiency.

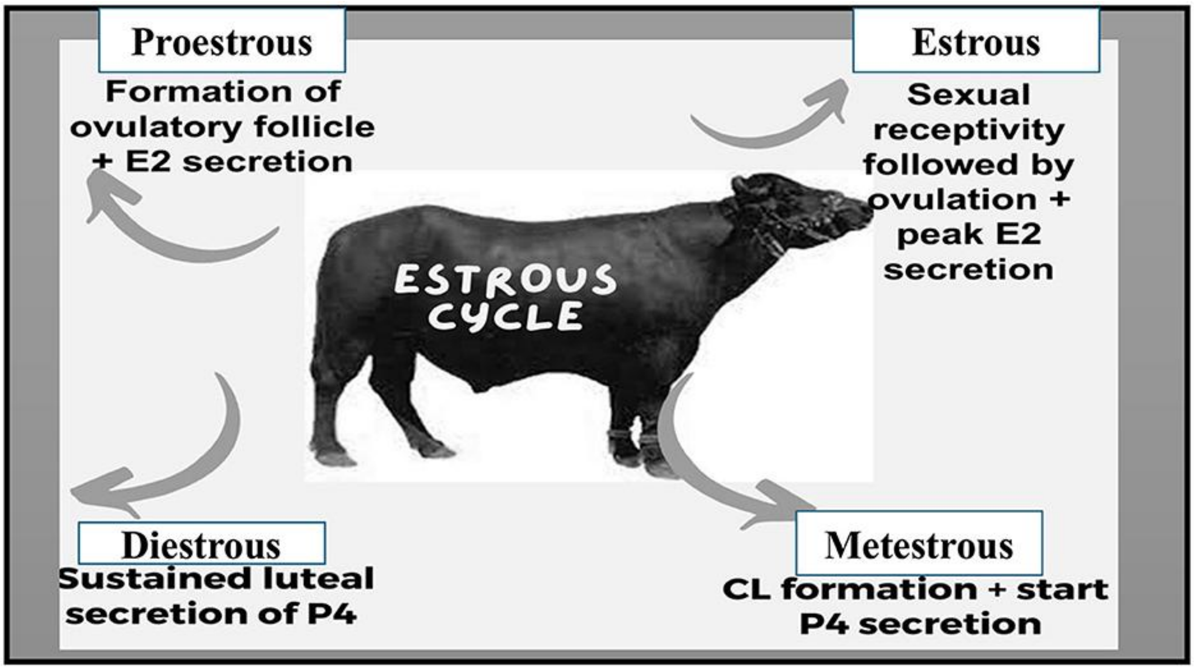

Figure 1 Sequential stages of the estrous cycle (Adopted from Kolachi et al., 2025)

5 Case Study Analysis

5.1 IVF application case at a buffalo breeding center in India

In India, the breeding of water buffaloes has actually been systematically advanced for a long time and is not a recent attempt. Artificial insemination technology was introduced as early as 1939, and later on, methods such as frozen semen and simultaneous estrus were continuously added. Interestingly, although technological tools are constantly changing, the goal has never changed: it is to find ways to improve genetic quality and milk production capacity. For instance, national-level projects such as the "Cattle and Water Buffalo Breeding Project" and the "National Dairy Cow Program" have made the promotion of artificial insemination more procedural. The average daily milk production has also increased from 3.4 kilograms in 1992 to 4.57 kilograms in 2009-2010. By 2010-2011, India was able to perform over 52 million artificial inseminations a year, and the average pregnancy rate remained at around 35%. Of course, it doesn't mean that everything has been settled. The current research focus has shifted to synchronous control and the improvement of semen quality. In vitro fertilization (IVF) and embryo transfer (ET) are also increasingly being used in breeding programs, with the aim of having more technical leverage when facing reproductive challenges (Singh and Balhara, 2016).

5.2 Performance and experience of AI promotion program in the Philippines

The development of water buffaloes in the Philippines has not been idle either, especially in the field of artificial insemination. They started early and did it quite solidly. The Philippine Buffalo Center (PCC) has simply integrated a complete set of timing artificial insemination (FTAI) strategies, such as CIR-Synch-HCG and the dual PGF2α protocol for summer use. These practices are mainly aimed at addressing the problems of difficult observation of estrus and low seasonal conception rates. In their gene bank farm, the effects of these programs were not bad - the conception rate rose to 52.6% in the summer of 2023 and was even higher in 2024, reaching 57.1%, while the control group remained at 27.3%. Behind this achievement, there is actually a set of supporting mechanisms: technicians need to be trained well first, and they also need to monitor reproductive indicators such as estrus signs and follicular size in real time before deciding on the timing of fertilization. The milk production also increased accordingly, indicating that this method not only works but also enables the farm to operate stably (Atabay et al., 2023; Fajardo et al., 2024; Maylem et al., 2025). However, then again, these good experiences cannot be replicated everywhere. After all, the success of artificial insemination is also related to the operator's level, animal management and even the weather (Ybanez et al., 2017).

5.3 Implementation and evaluation of OPU-ET trials in Southern China

In southern China, breeding techniques are becoming increasingly refined, especially the combined application of OPU-ET and IVF. On the experimental platform, many hybrid and purebred water buffaloes have adopted these methods. The improved concurrent estrus protocol was not proposed out of thin air. The approach like GPGMH, which combines mifepristone and hCG, is an adjustment based on the original Ovsynch. According to the data, the pregnancy rate brought about by this approach can be as high as 47.1% (Figure 2) (Abulaiti et al., 2021). Of course, the effectiveness of synchronous strategies like Ovsynch is not solely determined by the sound of the protocol name. Meta-analysis results show that the pregnancy rate of artificial insemination can be stably maintained between 42.6% and 46.4%, provided that the water buffaloes are operated during their estrus cycle. If it is during the non-estrus period, don't expect such a good outcome (Du et al., 2021). On the other hand, the use of follicle-stimulating hormone (FSH) before oocyte retrieval can significantly increase the quantity and quality of oocytes, which in turn increases the embryo production rate and improves the development of blastocysts (Sakaguchi et al., 2019). Overall, these technological combinations have significantly enhanced the efficiency of genetic improvement for water buffaloes and opened up new avenues for addressing the long-standing problem of low reproductive capacity.

6 Challenges in Technology Dissemination and Management

6.1 Cost of technology, equipment, and technical staff training

Ultimately, for assisted reproductive technology (ART) to truly take root, money is an unavoidable issue. Whether it is timed artificial insemination (FTAI), in vitro embryo transfer (IVEP), or embryo transfer (ET), they all rely on a complete set of equipment, hormones, and a well-equipped laboratory environment. None of these are cheap, especially for small-scale farmers or those in developing areas, the burden is quite heavy. In some places, even basic operation sites cannot be set up, let alone well-trained veterinarians or technicians. Even if there are people, if the skills are not up to par, it is hard to guarantee the effectiveness. Training is one thing, but continuous capacity building in the future must not be interrupted. In this way, the high technical threshold and large investment directly restrict many countries from making ART large-scale and sustainable (Baruselli et al., 2018; Srirattana et al., 2022).

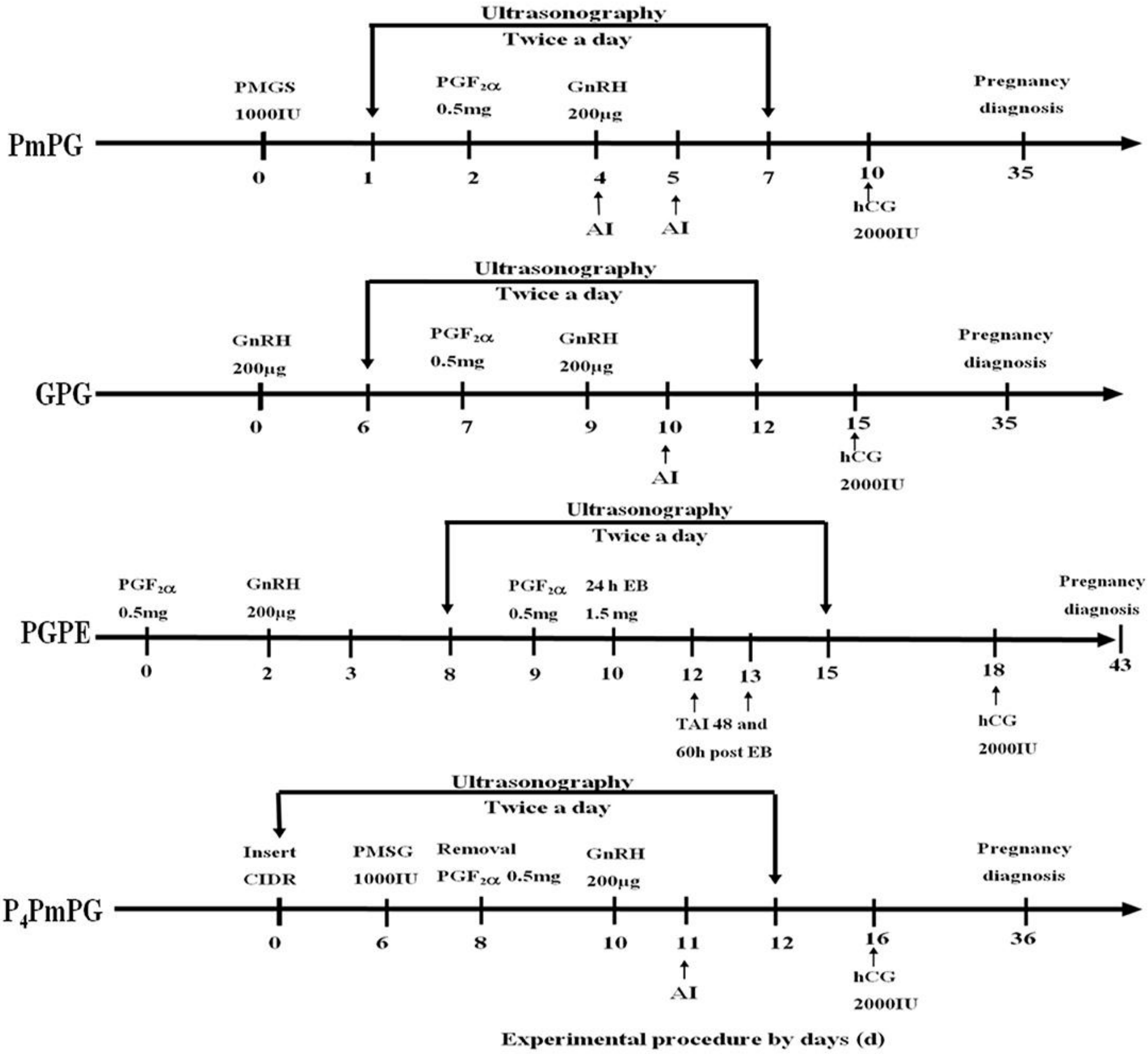

Figure 2 Schematic diagram of four conventional synchronization-TAI protocols (P4PmPG, GPG, PGPE, and PmPG) used in crossbred buffaloes during breeding season. The two downward vertical arrows in the window present the ultrasonographic observations of buffaloes synchronized through P4PmPG, GPG, PGPE, and PmPG protocols (Adopted from Abulaiti et al., 2021)

6.2 Farmer acceptance and conflicts with traditional husbandry practices

Not all farmers buy into the "new technology" as soon as they hear it, especially those family farms that have long relied on natural mating to raise cattle. For them, suddenly changing the approach - using hormones, doing synchronization, registering for check-ins, or even arranging laboratory operations - sounds strange and troublesome. They are worried about failure, spending money in vain and possibly disrupting the original rhythm. Some people prefer a conservative approach, even if it is less efficient, they still think it is safer. So, blind promotion is not enough; one must also find the right approach. Through on-site demonstrations, allowing them to see the results with their own eyes, and then combining the actual business model to design the plan, the acceptance level of farmers is likely to increase (Baruselli et al., 2023; Coman et al., 2024).

6.3 Establishment of data recording and reproductive performance evaluation systems

There is another issue that is often overlooked but very important: record-keeping. Assisted reproductive technology emphasizes efficiency and comparison, but without a clear data system, nothing can be evaluated. When does it come into heat? Which plan was used? How is the conception going? Does it have any impact on animal health? All of these have to be written down and calculated. The problem is that in many places, a standardized record-keeping method for water buffalo farming has not yet been established. A lot of data is based on experience or not recorded at all. This makes it very difficult to review the technical effects and to start optimizing the plans. To truly make ART a sustainable means of production, establishing digital systems and national databases is a lesson that must be made up sooner or later (Nava-Trujillo et al., 2020).

7 Conclusions and Future Perspectives

The changes in the water buffalo industry over the years have not been entirely sustained by traditional breeding. Assisted reproductive technologies (ART) such as timed artificial insemination (FTAI), egg retrieval (OPU), in vitro embryo transfer (IVEP), and embryo transfer (ET) have indeed made reproduction more "controllable". Especially for those situations where estrus is not obvious, seasonal influences are significant, or the natural conception rate is always low, ART offers many opportunities for solutions. Nowadays, not only have the levels of milk and meat production improved, but even gender can be "arranged" in advance. The speed of the spread of high-quality genes has also increased by more than a little compared to the past. Not to mention, measures such as gene banks and cryopreservation are gradually building up the safety net for the entire variety resources.

But then again, not all problems have been solved. The ovarian structure of water buffaloes is rather unique, which has also led to a persistent low efficiency in superovulation and embryo recovery. Methods like multiple ovulation - embryo transfer (MOET) are still not very realistic to be widely adopted. And don't forget, developing these technologies requires a lot of money, as well as technical personnel and equipment - for some small-scale farmers, the threshold alone is quite high. Not to mention, details such as the performance of different donors, breeding bulls, venue conditions, and feeding management will also directly affect the results. Stability is not always something that can be achieved at will. In addition, more "cutting-edge" tools such as somatic cell nuclear transfer (SCNT) or genomic selection are still in their infancy in most places. To truly put them into use, more experiments and cooperation are still needed.

As for the next direction, many people have already begun to try to combine genomic selection with ART for use. After all, finding promising and good seed sources as early as possible is the key to precision breeding. The collection of donor sperm before puberty, gender sorting, and the combination with automated reproductive systems, although they sound a bit futuristic, have gradually entered the practical stage. In the future, if efforts can be made in expanding the coverage of embryo transfer, developing automation, and using big data to manage reproductive effects, the overall efficiency will be greatly enhanced. Of course, this is not something that can be accomplished by a single laboratory or farm. Especially in regions like Southeast Asia, research cooperation networks, technology sharing and regional collaboration will be the key links to truly put these technologies into use.

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