Expression and Role of PIWI Proteins and piRNAs in Reproduction of Water Buffalo (*Bubalus bubalis, Linn.*)

Rocelle Joy C. Hufana[1], Perry Lorraine D. Canare[1], Evaristo A. Abella[1], Peregrino G. Duran[2,3], Rakesh Kumar[4] and Danilda Hufana-Duran[2,3,*]

[1]*Department of Biological Sciences, College of Science;* [2]*Department of Animal Science, College of Agriculture, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines*

[3]*Reproduction and Physiology Section, Department of Agriculture-Philippine Carabao Center, Science City of Munoz, Nueva Ecija 3120 Philippines*

[4]*Animal Biotechnology Centre, ICAR-NDRI, Karnal, India*

Abstract: High-fertile and productive dairy animals are important to satisfy the growing population's demand. Sire fertility is one of the essential factors that regulate the overall pregnancy rate of dairy herds. However, sire fertility varies from 10 to 90%, suggesting that male fertility largely accounts for varying fertility levels across the herd. Sub-fertile bulls and females should be identified and discarded promptly to improve the dairy herd's productivity. The most dominant factors implicated in culling are poor semen quality, poor semen freezability (<35% post-thaw motility), and poor libido for the bulls and hard breeders for females that cause huge economic loss to the raisers. Understanding the basic mechanism of male and female fertility has undergone tremendous change in recent times owing to the advancement of molecular tools judging the essential molecules responsible for fertility. Presently, a new molecular niche has surfaced in testes, strongly influencing the fertilization potential of spermatozoa. Over the last decade, there has arrived a conclusion that out of several factors, piRNA and PIWI proteins are largely implicated in regulating the vital aspects of fertility and embryogenesis. While this development is advancing in other animals, very limited information is available on PIWI protein and piRNAs in large animals. Except for a few sporadic information on PIWI protein in cattle, very limited information is available on piRNAs and PIWI protein in regulation with buffalo bull fertility and growth of embryos of buffaloes, posting a huge demand for research.

Keywords: PIWI proteins, piRNA, buffalo fertility, gametogenesis, reproduction, breeding.

INTRODUCTION

The world population of buffalo was nearly around 201 million in 2017 [1]. Water buffalo has a key role in the agricultural economy of many developing countries by providing milk, meat, and other by-products. Meat and dairy products are important sources of nutrition for many people around the world. Meat contains various nutrients such as protein, vitamin B12, and iron, while dairy products provide calcium and protein [2]. Meat production is a significant part of the global economy, contributing to local, national, and international trade. As consumers in developing countries gain more purchasing power, meat demand rise, giving a valuable source of high-quality protein in many people's diets [3] a huge challenge to produce. Meat production has more than tripled in global demand for over 50 years. Accounting for 40-45 percent of the worldwide meat supply, Asia is the world's largest meat producer [4].

On the other hand, milk production helps support family income, nutrition, and food security. It also provides small-scale producers with speedy profits and a vital source of income. In 2018, global milk output surged by more than 59 percent over the previous three decades. Since the 1970s, most expansions in milk production have been in South Asia, particularly in India, which is known as the world's largest milk producer, with 22 percent of global production [5,6].

Reproduction success is where producers' economic existence depends on, which has an impact on the price of meat and other animal products [7]. Reproductive failure is one of the most common causes of culling buffalo and cattle dairy cows and limiting the average number of milking lactations. Dairy animals must be known for efficient reproduction. However, they are affected by environmental factors, diet, producers' socio-economic circumstances, adaptability and genetic traits, and type of production system [6]. Despite having many merits of buffalo rearing, the reproductive disorders that include poor estrous expression, delayed onset of puberty, longer postpartum ovarian quiescence, and the most strikingly lower conception rate when bred through artificial insemination (AI) are the greatest limiting factors. The reproductive performance of female water buffaloes has been explored to a large extent; however, the assessment of buffalo bull fertility still needs to be discovered. To improve the overall productivity of the

*Address correspondence to this author at the Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines; Tel: +639171789002;
E-mail: danilda.duran@pcc.gov.ph, dhduran@clsu.edu.ph

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dairy herd, sub-fertile bulls should be identified and discarded quickly. Therefore, it is important to determine the low fertility factor before the animal is separated from the herd. Poor semen quality, poor semen freezability (<35% post-thaw motility), and poor libido are the most common factors for the culling down of bulls which causes a significant economic loss to the raiser [8,9].

Understanding of the basic mechanism of male and female fertility improved tremendously in the present times. Recently, a new molecular niche has been discovered in the testes, which strongly influences the fertilization of potential spermatozoa. PIWI or PIWI proteins and PIWI-interacting RNAs (piRNA) are largely implicated in the vital aspects of fertility and embryogenesis [10]. PIWI is an abbreviation of P-element Induced Wimpy testis in *Drosophila* [11]. PIWI-interacting RNAs, or piRNAs, are a collection of short non-coding RNAs interacting with the PIWI protein subfamily of the ARGONAUTE family. They are generally 24-32 nucleotides long and comprise at least hundreds of thousands of species [12]. Although several mechanisms have been identified, there is still least information regarding the biogenesis of piRNAs in water buffaloes. The piRNAs have a notable strand influence, meaning single strands of DNA are formed, which might mean they result from lengthy single-stranded precursor molecules [13,14]. A 'Ping Pong' process is also proposed, in which initial piRNAs recognize their complementary targets and recruit PIWI proteins. This causes the transcript to be cleaved ten nucleotides from the main piRNA's 5' end, producing the secondary piRNA [15]. These second piRNAs are specifically focused on sequences with adenine at the tenth position [16]. The ping pong cycle only works at the transcriptional level since the piRNA implicated in the cycle targets transposon transcripts [17]. In different species, one or both of these processes may be active; *Caenorhabditis elegans*, for example, does have piRNAs but does not appear to apply the ping-pong mechanism at all [18]. A large number of piRNAs have been discovered in zebrafish and *Drosophila melanogaster* containing adenine at their tenth position, and this has been cited as evidence of a conserved metabolic route across species [19,20]. However, the evidence is still to be reported on water buffalo. It has been discovered in very ancient species, such as sponges and cnidarians, that the ping-pong cycle existed even in the earliest metazoans [21] because of the wide variation in piRNA sequences and PIWI function among species, the challenge in determining the functioning of piRNAs increases [22]. However, like other small RNAs, piRNAs are thought to be involved in gene silencing, specifically the silencing of transposons [23]. Because most piRNAs are complementary to transposon sequences, which likely the transposons target piRNA, it appears that the activity of piRNAs in transposon silencing is most significant during the embryo's development. In *Caenorhabditis elegans* and humans, piRNAs are necessary for spermatogenesis and are implicated in all mammals [13,16,17,22] though no evidence has yet been reported in water buffalo. According to Litwack [24], one million copies of piRNA are present in spermatocyte or spermatid cells, found in the nucleus and cytoplasm.

This review article aims to encapsulate the recent progress of research regarding PIWI Protein and piRNA in different species of animals with emphasis on water buffalo. It focuses on the vital aspects of fertility and embryogenesis and their role in the fertility of *Bubalus bubalis*.

2. ROLE OF PIWI PROTEIN AND PIRNA IN MALE REPRODUCTION

Each year, the ability of a buffalo cow or heifer to reproduce, conceive, deliver, and raise a healthy calf is essentially important for successful and profitable production. The fertility of the female is related to the fertility of the parent bull; hence, understanding the male reproduction's anatomy and physiology is important for successful reproduction management [25]. Even though buffalo can adapt to harsh environments and live on low qualify forage, their reproductive effectiveness is frequently compromised by such conditions [26]. Understanding the molecular mechanisms behind fertility is paramount in improving reproduction performance. Table **1** presents the roles of PIWI proteins and piRNAs in male reproduction. While reproductive functions of PIWI proteins and piRNA are documented, research in water buffalo is absent. Preliminary studies were initiated [27] to explain the biogenesis and interaction of piRNAs and PIWI proteins in buffalo testes in relation to bulls' fertility, but the project is still ongoing, and results are yet to be published.

Germline Development

The formation of cell lineage that gives rise to the reproductive cells, known as gametes, of sexually reproducing animals is defined as germline development [45]. Understanding the germline development in farm animals is critical, especially in low-breeding bubaline species [46]. Current researches

Table 1: PIWI Proteins or PIWI Orthologs are Associated with Reproduction-Related Functions in Different Species

S.No.	Protein	Model/Species	Reproductive Functions	References
1.	PIWI	*Drosohphila melanogaster*	**Female**: Eliminate self-renewing division of germline stem cells (GSCs), mispositioned GSCs at the onset of oogenesis	Cox *et al*., 1998 [28]
			Female: Reduced GSCs division	Cox *et al*., 2000 [29]
			Embryos: Affects localization of OSK and VASA.	Megosh *et al*., 2006 [30]
2.	MILI	*Mus musculus*	**Male**: Blockage of spermatogenesis during early stage prophase stage of meiosis (from zygotene to early pachytene) rendering mice sterile, Failure of spermatocyte differentiation	Kuramochi-Miyagawa *et al*., 2004 [31]
			Male: Failure of spermatogenesis	Aravin *et al*., 2006 [32]
			Male: Complete sterility, spermatogenic arrest during meiosis	Aravin *et al*., 2008 [16]
			Male: Failure of GSCs renewal and differentiation	Unhavaithaya *et al*., 2009 [33]
3.	MIWI	*Mus musculus*	**Male**: Spermatogenic arrest at the beginning of round spermatid stage	Deng and Lin, 2002 [34]
			Male: Failure of spermiogenesis	Grivna *et al*., 2006 [35]
4.	MIWI2	*Mus musculus*	**Male**: Meiotic progression defect in early prophase of meiosis I and a marked progressive loss of germ cells with age.	Carmell *et al*., 2007 [36]
			Male: Genome instability and improper gametogenesis	Aravin *et al*., 2008 [16]
			Male: Failure of DNA methylation of fetal male germ cells	Kuramochi-Miyagawa *et al*., 2008 [37]
5.	ZILI	*Danio rerio*	**Male and female**: Failure of germ cell meiosis	Houwing *et al*., 2008 [38]
6.	ZIWI	*Danio rerio*	**Male and female**: Failure of germ cell meiosis	Houwing *et al*., 2008 [38]
7.	SMEDWI2	*Schmidtea Mediterranean*	Regeneration defect	Reddien *et al*., 2005 [39]
			Defective homeostasis in the germ line	Palakodeti *et al*., 2008 [40]
8.	SMEDWI3	*Schmidtea Mediterranean*	Regeneration defects	Reddien *et al*., 2005 [39]
			Defective homeostasis in the germ line	Palakodeti *et al*., 2008 [40]
9.	TWI1P	*Tetrahymena thermophila*	Conjugation defects	Mochizuki and Gorovsky 2004 [41]
			Defects in parental and developing new macronuclei	Aronica *et al*., 2008 [42]
10.	HIWI	*Homo sapiens*	**Male**: Testicular tumors	Qiao *et al*., 2002 [43]
			Germ cell malignancy	Liu *et al*., 2006 [44]

provide evidence about PIWI proteins and piRNAs functionally conserved in the germline development of different organisms [12]. PIWI family proteins are important for germline development and bind PIWI-interacting RNAs (piRNAs 1 2 3) [47]. PIWI proteins occur in many forms and work at different stages of the germline cycle. From the germline development's early phases (germline fate specification) to the late stages of gametogenesis, PIWI proteins play a critical function in various animals [10]. PIWI proteins have been shown to play a function in germ cell determination during early development [48]. Germ cell development

requires a particular kind of germplasm necessary and sufficient for the formation of germ cells in the embryo's posterior pole. During oogenesis, similar electron-dense structures can be observed at various stages of germline development, in particular as the Nuage, a perinuclear germline structure. In many metazoan species, including p granules, a class of perinuclear RNA granules specific to the germline have similar germline structures in *Caenorhabditis elegans* [49], the mitochondrial cloud in *Xenopus*, and the chromatid body in mammalian testes [45].

DICER1 and dFMRP were discovered to be linked to PIWI [29]. DICER1 is needed for miRNA biosynthesis and function, while dFMRP plays a direct role in the said processes [50,51]. Depletion of dFMRP or DICER1 similarly decreases germ cell development, which is consistent with the function of maternal PIWI and knowledge of germ cell-specific miRNAs [29]. Furthermore, the PIWI protein interacts with a limited number of miRNAs as well as a significant number of piRNAs [52]. All of these findings point to the miRNA process as the mechanism through which PIWI controls the development of primordial germ cells.

In larger animals like cattle and buffalo, ejaculated bovine semen contains small RNAs associated with sperm defects and reproductive problems. To give additional quality measurements for cryopreserved semen used for breeding, a technique based on deep sequencing of sperm microRNA (miRNA) and PIWI-interacting RNA (piRNA) from individual bulls was developed [53]. These authors identified 83 miRNAs and 79 putative piRNAs and found that gene pathways targeted by 40 known differentially expressed miRNAs were related to apoptosis. Table **2** presents the piRNAs associated with reproductive functions in different species.

Semen Quality

Methods for assessing sperm quality are critical for estimating the capacity of preserved spermatozoa to fertilize and enhance animal reproductive technologies [62]. Some studies show that piRNAs may have a potential sperm quality potential in swine and other farm animals like water buffalo [63]. The roles of piRNA's partner PIWI proteins, such as MIWI, MIWI2, and MILI, in stem cell self-renewal and the development of male germ cells in spermatogenesis confirms piRNA's significance [28]. The apiRNA analyzes the piRNA component of the porcine sperm and characterizes the connection between piRNA abundance and semen quality traits. The abundance of individual piRNAs has a beneficial effect on semen quality. Although none of the correlations attained significant levels, this overall approach was discovered when examining total piRNA content and semen quality parameters [63]. This means that the decrease in the quantity of piRNA leads to a decrease in semen quality. This is due to the lack of silencing of Receptor Elements or RE silencing and the associated increase in genome instability, which might have an impact on germ cell development [64,65].

Undernutrition-induced testicular regression is an interesting experimental method for learning more about the roles of piRNAs in spermatogenesis [66]. It has been recognized that 35 putative piRNAs, including oar-piR-12568 and oar-piR-9006, were linked with sperm production and quality in both well-fed and underfed males. In testicular tissue of underfed and well-fed male ovine, there was a positive connection between the proportions of miRNAs and putative piRNAs, indicating a synergistic relation between classes of small RNAs, a hypothesis that should be tested in further studies. Moreover, unlike the miRNAs, piRNAs are not conserved in several species.

Libido and Fertility

Bull libido, or sex drive, is a measurable feature with a large genetic component. It represents the main aspect of bull reproductive performance, with positive results in the herd's pregnancy rates and patterns [67]. Several independent studies show that Small non-coding RNAs (sncRNA) play a role in the molecular pathways that control spermatogenesis, testes development [68,69], and fertility [70]. Traditional semen and hormone analysis provide only limited information on lower reproductive potential, and male subfertility is frequently associated with poor health. Utilizing the identification of a suitable combination of in -vitro sperm function was developed for the prediction of fertility in buffalo bulls [71]. The transcriptomic profile of buffalo spermatozoa revealed dysregulation of functionally relevant mRNAs in low-fertile bulls [72]. Small non-coding RNAs (sncRNAs) circulated in the bloodstream are paracrine and endocrine messengers with prognostic value. PIWI-interacting RNAs (sncRNAs) are a common single-stranded type of small non-coding RNAs (sncRNAs) that protect genomic integrity in the germline by acting against transposable elements which they were first found in gonadal cells [73]. Abnormal epigenetic programming of the germ line is proposed as a possible mechanism for

Table 2: piRNAs Associated with Reproductive Functions in Different Species

Sl. No.	piRNA	Model/Species	Reproductive Functions	References
1.	piR-1207, piR-2107, piR-5937, piR-5939	*Homo sapiens*	Asthenozoospermia	Hong *et al*., 2021 [54]
2.	DQ601609, DQ589977 DQ591415, DQ5989918	*Mus musculus*	Male infertility	Wu *et al*., 2020 [55]
3.	piR-52207 piR-3373	*Homo sapiens*	Ovarian cancer	Wu *et al*., 2020 [56]
4.	hsa-piR-20830, hsa-piR-4731, hsa-piR-6254 hsa-piR-419, hsa-piR-7152, hsa-piR-7548 hsa-piR-14195, hsa-piR-5026, hsa-piR-11482 hsa-piR-17765, hsa-piR-17102, hsa-piR-4484, hsa-piR-17260, hsa-piR-17098, hsa-piR-20511 hsa-piR-5802, hsa-piR-2510, hsa-piR-19121 hsa-piR-4745, hsa-piR-11873	*Homo sapiens*	Obstructive azoospermia	Cao *et al*., 2018 [57]
5.	Chromosome 18 pachytene piRNAs	*Mus musculus*	Decreased spermatogenesis and male fertility, sperm acrosome overgrowth	Choi *et al*., 2021 [58]
6.	piR-170840 piR-604682	*Mus musculus*	Essential for sperm formation	Zhang *et al*., 2015 [59]
7.	piRNAs produced from pi6 locus	*Mus musculus*	Reduced male fertility, smaller litter size, reduced capacity of sperm to fertilize eggs during IVF and ICSI, Difficulty in zona pellucida penetration, impaired capacitation, Failure of embryo development,	Wu *et al*., 2020 [55]
8.	piRNA complex	*Caenorhabditis elegans*	Required for Fertility	Batista *et al*., 2008 [60]
9.	piRNA	*Sus scrofa*	Cumulus-oocyte complex and early embryos	Yang *et al*., 2012 [61]

compromised spermatogenesis [74]. Studies in mutant PIWI proteins enlightened their role in spermatogenesis and fertility. In recent years, the biological complexity of sperm has become more evident with the discovery of a rich sperm RNA population with functional roles in fertilization [75]. Mature sperm RNAs have been studied in several mammalian species, including cattle [76]. The sncRNA population of sperm has been interrogated in several mammals, which is composed of a large and complex repertoire of piRNAs and other RNA classes [77].

All seminal plasma pRNAs were shown to be downregulated in infertile individuals in recent research [78]. Another analysis revealed that in male factor infertility, the expression of certain sperm piRNAs (has-piR-31704 and has-piR-39888) declined and had a relation to sperm counts and fertilization rate [79]. Due to the distinct profile of piRNAs found in seminal plasma and serum, these specific piRNAs were not identified. As a result, understanding the piRNAs' potential function is difficult to anticipate. Indeed, there is no publication or other functional information on hsa-piR26399. However, because piRNAs are tissue-specific, they may be even more selective biomarkers for infertility, underlining the potential value of the discovered hsa-piR-26399 [80].

3. ROLE OF PIWI PROTEIN AND PIRNA IN FEMALE REPRODUCTION

Female reproduction of water buffalos normally produces calves every other year. Successful fertilization and subsequent embryo development depend on the complex molecular procedure that begins with the maturation of the oocyte and preserving its competence through development. MicroRNAs or

miRNAs are small non-coding RNA molecules used as gene regulators in several biological systems, as well as oocyte and embryo [81].

Expression in Oocytes and Early Embryos

Most animals' germ cells rely heavily on piRNAs and PIWI proteins. The piRNAs that mimic testis-borne pachytene piRNAs have been discovered in big animals, including bovine ovaries. It was discovered that isolated bovine follicular oocytes contain a large number of small piRNAs that selectively target transposable elements. A label quantitative proteome analysis revealed that mature oocytes express the PIWIL3 protein, as well as other known piRNA-pathway components, significantly and selectively. Early bovine embryos still have a piRNA pool, indicating that piRNAs may influence mammalian development. This demonstrates that piRNA pathways in the oocytes of mammals and early embryos are very dynamic [82]. The observed expression pattern shows that PIWIL3 has a function throughout meiosis and pre-implantation development but not during the blastocyst stage's separation of embryonic and extra-embryonic cell lineages. Expression levels in morula and blastocyst samples were either undetectable or did not exceed the background levels of their Reverse Transcription controls. This result demonstrated that PIWIL3 does not play a significant role at this point. When MII oocytes were compared to GV-stage oocytes and zygotes, there was a considerable, but not significant, up-regulation of PIWIL3 transcripts, indicating that PIWIL3 may play a role in oocyte maturation and meiosis [83].

The testis also contained a complete PIWIL1 transcript and protein, especially in the germ cells of mature seminiferous tubules. Amplification of their distinct intronic segments verified the presence of truncated PIWIL1 isoforms in oocytes and testes. The early embryogenesis expression profile of PIWIL1 revealed maximal mRNA expression at the 2-cell stage, with levels decreasing as the blastocyst develops. PIWIL1-YFP fusion plasmids were produced for each isoform and expressed in HEK 293 cells to demonstrate nuclear exclusion and size-specific banding of the various isoforms. These findings are the first complete analysis of PIWIL1 in bovine, demonstrating functional similarities with PIWIL1 in other species and tissue-specific expression of several isoforms [84]. The piRNA pools were discovered to be targeted at or derived from certain mRNA arrangements. The number of piRNAs targeting mRNAs and their fold change in expression was shown to have substantial negative associations as seen in the characterizations of the PIWI/piRNA pathway in the translational bovine model, as well as in the unique environment of embryogenesis [85]. The role of miRNAs in the regulation of lactating physiology in the buffalo mammary gland is also demonstrated [86], and during pregnancy, changes in miRNA expression were also observed in the buffalo corpus luteum [87].

Reproductive Regulations and Function

In *Caenorhabditis elegans*, it has been discovered that the binding of PIWI protein to piRNA, which is non-coding, is critical for both reproductive and developing processes. Because mitochondrial function regulates piRNA biosynthesis, the relationship between mitochondrial activity and piRNA expression is anticipated to perform an unknown but crucial role in reproductive and developmental processes, as both are known to be influenced by the mitochondrial quality and activity declines [88].

Sex hormones, including estrogen and testosterone, control the piRNA pathways [89,90]. Estrogen and testosterone are sex hormones that regulate the piRNA pathway [91,92]. Therefore, the piRNA pathway may be affected by ovarian stimulation. Studies on the effect of different doses of pregnant mare serum gonadotrophin/human chorionic gonadotrophin and repeated ovarian stimulation on the expression of the Mili and Miwi showed that Miwi and Mili gene levels decreased significantly after treatment with 12.5 I.U. These results suggested that exogenous gonadotropin administration leads to a significant decrease in the expression of the Mili and Miwi which are critically important in the piRNA pathway. New comprehensive studies are needed to reduce the potential effects of ovarian stimulation on the piRNA pathway which silences transposable elements and maintains genome integrity. Exogenous gonadotropin administration may raise the risk of genetic instability, depending on the dose and number of repetitions [93].

4. STUDIES ON PIWI PROTEIN AND PIRNA IN WATER BUFFALO

In water buffalo, very limited studies have been published on PIWI protein and piRNA, showing that understanding the role of PIWI protein and piRNA in water buffalo reproduction is at the beginning stage. Studies on protein ubiquitination in water buffalo, a major and conserved post-traditional modification known to play a critical regulatory role in many

biological processes in eukaryotes, showed that PIWIL1 plays an important role in spermatogenesis. It was identified as one of the 38 ubiquitinated proteins that interact with diverse pathways during sperm cell development [94]. Ubiquitination was shown to regulate the morphogenesis and function of sperm organelles [95] and is involved in the regulation of cellular processes like DNA damage repair [96], DNA replication [97], receptor endocytosis, and innate immune signaling [98,99]. The identification of the PIWIL1 as one of the ubiquitinated proteins involved in spermatogenesis suggests that PIWIL1 is involved in the dense protein interaction networks and the coordination and cooperation lead to physiological function in buffalo testis [94].

According to Jerome *et al.* [87], piRNAs from corpus luteum or CL of early pregnancy confirms direct germline regulation, which is identified in the testis of rodents and pig species were confirmed to play a role during spermatogenesis [100,101]. However, the relevance of these piRNAs in the silencing of transposon in the CL of pregnant buffalo is yet unknown. In both neonatal and adult pig ovaries, piRNAs have been identified in ovarian tissue, with neonatal ovaries having a higher quantity of piRNAs than adult ovaries [102]. The piRNA regulation mechanism studies are still in their early phases. The piRNAs were identified as intergenic, intronic, and exonic piRNAs by in silico analysis. Furthermore, the majority of piRNA sequences determined were between 24-31 nts, showing that dicer does not process piRNAs.

In embryos and oocytes of bovine, the single-stranded RNA molecules are RNAs that attach to targets of RNA in redundancy and inhibit objectives either cutting or recruitment of chromatin mark. During genomic remodeling, Transposable Elements (TEs) were inhibited by piRNA both post-transcriptionally and transcriptionally [82]. It was first discovered to play a role in gametogenesis, and it has since been proven to inhibit TEs during altering events in the germline's primordial cell development [103]. However, the function of piRNA silencing of TEs during embryogenesis is undetermined. It has been observed that transcriptional activation of TEs occurred during the Maternal- Zygotic Transition or MZT. It was first recognized to play a function in gametogenesis, and it has since been demonstrated to inhibit TEs during primordial germline cell development reprogramming events [104]. Its role in gametogenesis was first discovered, and it has since been shown to suppress TEs during the events in the reprogramming of primordial germline cell development [103]. Other species, including humans, have been reported to have relatively high TE expression during the MZT [105]. Prior to the MZT's major wave, elevated expansion of piRNAs may be required as epigenetic programming occurs, inhibit TE activation, and maintain genomic integrity. The piRNAs target mRNA also showed degradation during pachytene spermatogenesis, suggesting that there may have another mechanism for suppressing gene expression in piRNA [106].

PIWI-interacting RNAs and high expression of PIWIL3 in bovine and PIWIL1 bovine oocytes have been detected [82]. Despite the fact that those results clearly establish the presence of the PIWI pathway in higher vertebrate embryos, the dynamics of PIWIL1, associated piRNAs, and any putative isoforms in buffalo testes, oocytes, and early embryos have yet to be thoroughly investigated. It has been evaluated that the discovery of two extra PIWIL1 isoforms as a result of bovine PIWIL1 expression in oocytes was tested for alterations during oocyte and embryo development. Although full-length PIWIL1 expression is restricted to the testis, two shorter PIWIL1 isoforms have been discovered and expressed in bovine oocytes and early embryos [84]. PIWIL proteins are required for piRNA target identification and degradation because they form complexes with piRNAs to control gene expression. Furthermore, a study that looked at the expression of piRNAs in cattle oocytes and zygotes discovered that the expression of some piRNAs appeared to be linked to genes that would be degraded in the embryo [85].

It has been pinpointed that the MZT in cattle embryos is most active at the 8-cell stage, with the first activation of genes involved in RNA processing happening at the 4-cell embryonic stage, kicking off large-scale embryonic genome activation (EGA) [107]. Small non-coding RNA (sncRNA) has a great potential to play a role in the degradation of maternal transcripts during the quiescence period due to its post-transcriptional control of several genes. MicroRNAs (miRNAs), piRNAs, and endogenous small interfering RNAs (siRNAs) are three forms of sncRNAs that have been revealed to have a role in early mammalian development. Although their biogenesis and targeting processes differ, all three sncRNA classes work through RNAi to degrade either mRNA or transposable element targets [108].

The N-terminal, PAZ, middle (MID), and PIWI domains are the four domains found in PIWI proteins;

of these, the PAZ and PIWI domains show the most conserved between species and when comparing members of the AGO family on an amino acid level. PIWI proteins' PAZ domain binds the 3′ termini of piRNAs, encouraging the 2′-O-methyl modification that is characteristic of mammalian piRNAs [109]. The AGO protein family's MID and PIWI domains recognize and bind the 5′ end of related short RNAs [110]. Recent MID-PIWI domain piRNA binding characterization revealed a bias towards sequences with 5'-undrine, as well as strand bias dependent on the PIWI family member [111]. The potential for promoter DNA hypermethylation-associated silencing of PIWI/piRNA pathway genes to diminish pachytene piRNA production during spermatogenesis in bovine spermatogenesis was also confirmed. The findings revealed that epigenetic changes to the entire PIWI/piRNA pathway cause bovine male infertility. This happened when male meiosis-specific genes were hypermethylated, and piRNA production was disrupted, resulting in failed germ cell formation and possible sterility in bovine hybrid males [112].

While it was earlier indicated that when male meiosis-specific genes were hypermethylated, piRNA production was impaired in bovine hybrid males, leading to unsuccessful germ cell development and probable sterility [85]. Very limited information was available on PIWI protein and piRNAs regarding large dairy animals whose fertility and reproduction are essentially important. Hence, studies are encouraged to understand the underlying function and role of these proteins in the regulation of reproduction in bovine, especially in water buffalo.

5. CONCLUSION AND RECOMMENDATION

Based on the data gathered, male and female fertility has been improving tremendously by a new molecular niche discovered in the testes, which is the PIWI proteins and piRNAs that strongly influence the fertilization of potential spermatozoa. It has largely been implicated in vital aspects of fertility and embryogenesis that occur from the earliest phases of germline development to the late stages of gametogenesis. PIWI controls the formation of primordial germ cells through the miRNA process. In semen quality, the reduction of piRNA abundance leads to a decrease in the quality of sperm due to a deficient silencing of Receptor Elements and the consequent increase of genome instability which could impact germ cell correct development. In male fertility, the biological complexity of sperm has become more evident with the finding of a rich sperm RNA population with functional roles in fertilization. In female reproduction, isolated bovine follicular oocytes were shown to have a high number of small piRNAs that target transposable elements selectively. The observed expression pattern indicates that PIWIL3 is active throughout meiosis and pre-implantation development but not during the separation of embryonic and extra-embryonic cell lineages in the blastocyst stage. Exogenous gonadotropin therapy has been shown in several studies to enhance estradiol levels, suggesting that ovarian stimulation may affect the piRNA pathway.

Despite the progress in male and female fertility in animal models, there still needs to be more information on Piwi protein and piRNAs in large dairy animals. Several studies are still needed for more information and knowledge about Piwi proteins and piRNA regarding water buffalo for effective reproduction to produce enough meat and milk for future purposes. There needs to be more information on the role of piRNAs and Piwi protein in bovine and cattle. Therefore, it is highly recommended to study more about Piwi proteins and piRNA in buffalo to understand their regulation and function on bull fertility and embryo development.

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