Review Article

Reproduction and *In Vitro* Technologies of Swamp Buffalo; Past, Present, and Future

Ruchikon Jongsuwanwattana[1,2] Theerawat Swangchan-Uthai[1,2] Sudson Sirivaidyapong[1*]

Abstract

Swamp buffalo (*Bubalus bubalis carabanensis*) has long been integral to agriculture and has served as an essential source of food security in Southeast Asia, particularly in Thailand. Despite their economic and cultural significance, swamp buffaloes have been at risk of declining populations due to low fertility rates. This review provides a comprehensive overview of swamp buffalo reproduction, examining past and present practices, identifying gaps in the literature, and exploring potential future directions for improving reproductive efficiency. This examination investigates the domestic buffalo's reproductive anatomy and physiology, providing a comparative analysis among ruminants. Recent advancements in assisted reproductive technologies offer substantial opportunities to enhance fertility, but the conception rate is still low (30 to 50%) in this species. Research on the cellular and molecular aspects of implantation and uterine receptivity in buffalo is still limited despite its critical importance for improving fertility, considering the high incidence of pregnancy loss during early gestation. An in vitro endometrial cell culture system offers insight into the maternal-embryonic crosstalk during this critical period. Understanding these intricate mechanisms is crucial for developing more effective reproductive strategies and accelerating genetic enhancement, thereby enhancing fertility rates and supporting the sustainability of swamp buffalo populations.

Keywords: fertility, reproduction, swamp buffalo, Thailand, uterine receptivity

[1]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*CU-Animal Fertility Research Unit, Chulalongkorn University, Bangkok, Thailand*
***Correspondence**: Sudson.s@chula.ac.th* (S. Sirivaidyapong)
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Introduction

The domestic water buffalo is primarily classified into two types by genetic selection: the swamp type (*B. bubalis carabanensis*; 48 chromosomes) and the river type (*B. bubalis bubalis*; 50 chromosomes). Over the past decades, swamp buffaloes have been domesticated in Thailand and have become a crucial livestock resource for developing countries. Swamp buffalo farming is widespread across Southeast Asia, often integrated with crop-livestock farming systems. Additionally, swamp buffaloes play significant roles in cultural events and religious rituals in some regional areas, contributing to the economy and improving the standard of living for agriculturists. They are also used for meat production, serving as an excellent source of protein. Meanwhile, the river type buffaloes, Murrah breed from South Asia and Europe, are mainly raised for milk production by smallholder farms. Both swamp and river buffaloes are essential for food security (Borghese *et al.*, 2022). The increasing significance of buffaloes necessitates an accelerated rate of genetic improvement to enhance production efficiency and sustainability.

Buffalo has traditionally been considered a species with low fertility, and declining birth rates have raised significant concerns (Gordon, 1996). However, several studies suggest that proper management and feeding can improve fertility under optimal environmental conditions (Usmani *et al.*, 1990; Qureshi *et al.*, 2007). Despite efforts to enhance reproductive performance in buffalo, including adoption of assisted reproductive technologies (ARTs) from cattle and other species, the results are still not as expected. Presently, limited evidence focuses on the cellular and molecular aspects of the implantation process and uterine receptivity in swamp buffalo. This area of research is particularly crucial for improving fertility due to the high incidence of pregnancy loss during early gestation. Comprehending the complex mechanisms behind uterine receptivity and implantation is vital for devising more effective reproductive strategies. This review aims to provide a comprehensive overview of swamp buffalo reproduction and synthesize current knowledge on these cellular and molecular aspects, highlight gaps in the literature, and suggest potential directions for future research to enhance fertility in swamp buffalo.

Overview of domesticated swamp buffalo in Thailand

Swamp buffalo is an economically important livestock in many developing countries, including Southeast Asian Nations. In Thailand, swamp buffalo have been habituated for many centuries, becoming one of the nation's native animals. Moreover, Thailand has the largest buffalo population in Southeast Asian countries. Domesticated swamp buffalo are primarily used for meat production, providing an excellent source of protein, and as draft animals in paddy fields, especially in rural agricultural areas. Swamp buffalo are highly valued for their ability to utilize poor-quality roughage from locally available natural resources and their capacity to adapt to harsh environments. Importantly, they resist various tropical diseases naturally (Chantalakhana, 1986). Rearing buffalo is a long-term capital asset for smallholders, protecting them from financial risks. The acceptability of buffalo meat is progressively increasing. Smallholders typically sell calves, non-productive buffaloes, and animal manure to alleviate financial crises. Buffalo contributes economically to smallholder farms through multiple benefits, enhancing the income of rural cultivators. However, due to social changes, modernization, and industrialization, the global buffalo population has declined considerably over the last decades (FAOSTAT, 2022). The global buffalo population is estimated to be approximately 200 million.

In Thailand, the buffalo population has experienced a significant decline, decreasing by 57% from around 3 million in 1997 to 1.7 million in 2023 (DLD, 2024). This population decline has led to the loss of valuable genetic characteristics, impacting fertility rates, as higher genetic buffaloes tend to have fewer offspring and lower breeding success (Roy and Prakash, 2009). Furthermore, there has been a lack of practical support policies for buffalo breeding programs over the last decades, with more attention given to cattle in livestock development due to agricultural economic considerations.

Fertility of swamp buffalo

Over the past few decades, researchers have studied reproduction in buffalo. The findings indicated that the reproductive anatomy and physiology of buffalo are similar to those of cattle (Taneja *et al.*, 1995; Warriach and Ahmad, 2007; Majarune *et al.*, 2023). Comparative studies have revealed significant aspects of reproductive anatomy. The pelvimetry of swamp buffalo is greater than that of cattle at similar body weights and ages, with a more circular pelvic inlet (Chantaraprateep *et al.*, 1984). The cervix of buffalo is smaller and has a narrower opening compared to cattle, while the uterus is smaller and coiled within the pelvic cavity (Perera *et al.*, 1987). Additionally, the ovarian contents during the estrous cycle have been documented, showing that the size of ovarian contents in buffalo is smaller than in cattle (Jainudeen *et al.*, 1983; Yindee *et al.*, 2011). Comparative studies between swamp and river buffalo indicate similar reproductive anatomy and physiology (Chantaraprateep *et al.*, 1984; Baruselli *et al.*, 1997; Perera, 2011). However, significant differences in reproductive performance have been observed. Swamp buffalo require more services per conception (9.89±3.29 vs. 8.05±4.24) and have a higher age at first calving (4.16±0.89 vs. 3.79±0.50 years) compared to river buffalo in the first parity (Kanloung *et al.*, 2021).

Swamp buffalo heifers typically attain puberty at 21 to 24 months of age, reaching 55 to 60% of their body weight (Jainudeen and Hafez, 2000). In Thailand, findings indicate that the average age of puberty in female swamp buffalo is 3 to 4 years (Bodhipaksha *et al.*, 1978). Puberty is attained at different ages between types, with river buffalo reaching puberty at 15 to 18 months (Borghese, 2005). The age of puberty is influenced by several factors, including genotype, nutrition, farm management, diseases or disorders, environment, and climate (Chantalakhana, 1986; Jainudeen and Hafez, 2000; Campanile *et al*., 2010).

Buffaloes are polyestrous animals, with an estrous cycle duration averaging 19.9±4.4 hours in the swamp buffalo (Kanai and Shimizu, 1983) and 23.8±6.2 hours in the river buffalo (Luktuke and Ahuja, 1961). The variability in the estrous cycle in buffalo is attributed to factors such as nutrition, ovarian steroid hormone secretion, and environmental conditions (Nanda *et al*., 2003). Estrus duration ranges from 5 to 27 hours, and ovulation occurs between 6 and 21 hours (Hafez, 1993; Oswin Perera, 1999; Perera, 2011). Additionally, the estimated interval from the end of estrus to ovulation is approximately 13.9 hours in swamp buffalo and 11 hours in river buffalo (Luktuke and Ahuja, 1961; Kanai and Shimizu, 1983). Signs of heat in buffalo, including restlessness, bellowing, heat mucus appearance, and physiological changes of the vulva, are similar to those in cattle but are generally more difficult to detect, especially during periods of high ambient temperature (Perera *et al*., 1987). Hormonal changes throughout the estrous cycle have been extensively reviewed and are re-illustrated in Figure 1 (Taneja *et al*., 1996; Baruselli *et al*., 1997; Yindee *et al*., 2011). The gestation period in buffalo ranges from 300 to 340 days. Parturition in swamp buffalo typically involves an uncomplicated labor and delivery process, although occasional dystocia cases have been reported. The stage of parturition is divided into three parts: the dilation of the cervix, the expulsion of the fetus, and the expulsion of the placenta, similar to other ruminants (Mathias, 1980; Senger, 2012).

Studies have reported that swamp buffaloes raised under intensive conditions exhibit low reproductive performance, including delayed age of first calving, higher service per conception, and prolonged days open in primiparous cows (Chaikhun *et al*., 2013). Reduced fertility in buffaloes is attributed to delayed puberty, diminished heat sign expression, prolonged postpartum ovarian inactivity, low and inconsistent response to hormonal stimulation for ovulation synchronization and artificial insemination, embryonic mortality, and poor conception rates (Terzano *et al*., 2007; Chaikhun *et al*., 2010; Chaikhun *et al*., 2013). Furthermore, the application of reproductive technologies such as artificial insemination, hormonal synchronization, and embryo transfer from related species has resulted in poor fertility outcomes (Gasparrini, 2002). A recent study found that pregnancy rates in buffalo with hormonal synchronizations range from 30 to 50% (Warriach *et al*., 2015). Implantation failure is a major cause of pregnancy loss among mammalian species. Evidence suggests that early embryonic death accounts for approximately 40% of pregnancy losses, which is three to four times greater than losses due to fertilization failure (Diskin *et al*., 2006; Diskin and Morris, 2008). Further research is needed to elucidate and enhance the understanding of swamp buffalo reproduction, aiming to improve the reproductive performance of this species. Several studies have utilized ART to enhance reproductive indices and preserve desirable genetic characteristics of swamp buffalo (Neglia *et al*., 2003; Selokar *et al*., 2018; Gutiérrez-Añez *et al*., 2022).

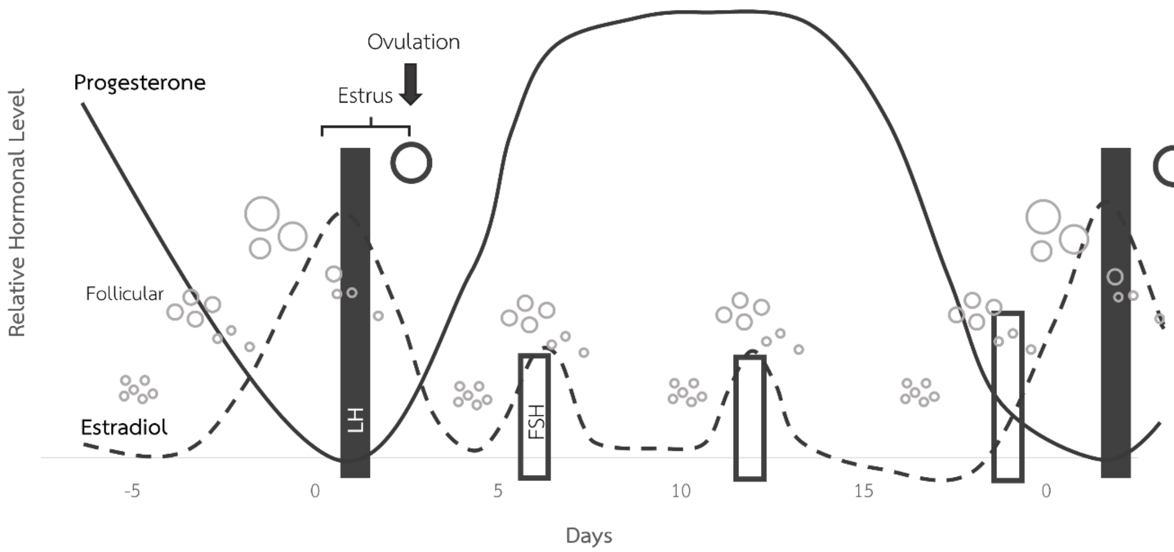

Figure 1 The hormonal changes of buffalo during the estrous cycle.

Embryo development

The duration of embryonic development among ruminant species has been distinguished in several studies, described in Table 1 (Chantaraprateep *et al*., 1989; Campanile *et al*., 2010; Brooks *et al*., 2014; Spencer and Hansen, 2015). Previous research has shown that the early stages of embryonic development in swamp buffalo are quite similar to those in cattle, with the notable exception that buffalo embryos develop approximately seven days earlier (Gasparrini, 2002). Specifically, buffalo embryos exhibit more advanced development by about 12 to 24 hours compared to cattle. This accelerated development is linked to their earlier uterine entry, which occurs around 4 to 5 days post-insemination. This earlier progression into the uterus might influence subsequent stages of pregnancy and overall reproductive success.

The establishment of pregnancy in ruminants begins at the embryonic development stage and implicates the maternal recognition of pregnancy signaling, implantation, and placentation (Spencer and Hansen, 2015). A coordinated sequence of events is necessary to establish and maintain a pregnancy. This complex process requires proper crosstalk and interaction between the maternal endometrium and the developing conceptus. For the successful establishment of pregnancy, the maternal endometrium must undergo specific developmental changes to allow the conceptus to attach during the window of implantation period (Nagaoka *et al*., 2000). Concurrently, the developing conceptus must reach the blastocyst stage and undergo elongation to initiate maternal-embryonic interactions. Inadequate or improper signaling from either the maternal endometrium or the developing conceptus, especially regarding maternal recognition of pregnancy signaling, can result in pregnancy failure (Bazer, 2013; Brooks *et al*., 2014). The *in vitro* co-culture studies between the maternal endometrium and embryo in buffalo showed that embryos co-cultured with steroid-treated endometrial epithelial cells significantly stimulated the relative mRNA abundance of interferon-stimulated genes (ISGs) (Huidrom et al., 2022). The finding also proposed pregnancy-associated glycoprotein-1 (PAG-1), interferon-tau (IFNt), and ISGs expression as diagnostic and prognostic markers of maternal-fetal cellular interaction in buffalo cows (Casano *et al*., 2023).

During this critical period, implantation failure has been identified as a primary cause of pregnancy loss and infertility, leading to substantial economic losses for producers. The loss of a developing conceptus within the first 42 days of pregnancy, termed early embryonic mortality, directly impacts reproductive performance. Diskin and Morris (2008) reported an embryonic and fetal mortality rate of approximately 40% in moderate-producing cows based on a fertilization rate of 90%. It indicates that reproductive losses in dairy cows due to early embryo death are three to four times greater than losses due to fertilization failure. These losses are particularly pronounced in repeat breeders, which are ruminants that have undergone three or more insemination attempts. The increasing rate of embryonic mortality is often attributed to improper physiological regulation of uterine function, usually occurring during the initial phases of gestation or the first 40 days of pregnancy (Hansen, 2002). In buffalo, early embryonic mortality is typically associated with the delay in the rise of progesterone secretion (Campanile *et al*., 2010). Furthermore, buffalo treated with prostaglandin F at the time of artificial insemination had a 16% greater pregnancy rate than untreated buffalo (Neglia *et al*., 2008), suggests that progesterone secretion is crucial for uterine function through embryonic development and the success of pregnancy. Additionally, there also reported that the gamete quality contributed to the fertilization outcome. Oocytes collected from buffalo in the summer-autumn transition had higher cleavage and blastocyst rates than those collected in the winter-spring transition (Campanile *et al*., 2005).

Reduced progesterone secretion appears to be a contributing factor to early embryonic loss. Buffalo females treated with hormones such as human chorionic gonadotropin (hCG), progesterone, or gonadotropin-releasing hormone agonist (GnRH agonist) after artificial insemination do not consistently exhibit lower embryonic mortality rates (Campanile *et al*., 2007). Several related species findings have also been described that early embryonic loss is associated with low circulating progesterone levels (Wilmut *et al*., 1986; Bilodeau-Goeseels and Kastelic, 2003). It is hypothesized that reduced progesterone secretion by the corpus luteum is a primary cause of embryonic loss. However, some researchers have found that approximately 50 to 60% of buffaloes experiencing embryonic loss maintain progesterone levels similar to those of pregnant buffaloes (Campanile *et al*., 2005). This suggests that progesterone alone may not be a reliable indicator of pregnancy status in buffaloes and that other factors might contribute to embryonic loss despite normal progesterone levels. Understanding these additional factors is crucial for improving reproductive management and reducing embryonic loss in buffalo populations. Exogenous hormone treatments are required to induce and elevate progesterone levels throughout the early development stage, embryonic attachment, and placentation. It has been reported that the use of hormonal treatments between 25 and 40 days post-insemination has only been effective in farms with high embryonic mortality rates due to the low efficiency of artificial insemination (Campanile *et al*., 2007). Although the treatments focused on lowering the embryonic mortality rate in cattle and other small ruminants have been successfully demonstrated, there are not yet methods appropriate for buffaloes, and further evidence is required to improve hormonal treatment protocols for applicability.

Uterine receptivity and pre-implantation period

During the pre-attachment stage, the spatial and temporal expression patterns of several factors are thought to facilitate communication between the maternal endometrium and the developing conceptus (Bauersachs *et al*., 2008). Successful implantation requires appropriate interactions between the blastocyst and uterus, involving molecules such as interferon-tau, progesterone, prostaglandin, and cytokines (Brooks *et al*., 2014). Proper secretion of interferon-tau and progesterone is crucial for initiating and maintaining the molecular signaling cascade necessary during the attachment period. Adhesive molecules and cytokines play a critical role in implantation, and their improper expression can lead to adverse pregnancy outcomes. Dysfunctional endometrial activity may result in embryonic mortality and reduced fertility (Spencer and Hansen, 2015). There has been interest in human embryo production, which can be incorporated into a model system for studying maternal-embryonic interactions in research and clinical aspects (Holmberg *et al*., 2012; Weimar *et* *al*., 2013). Somehow, knowledge about implantation failure in buffalo species is limited, necessitating a better understanding of embryo implantation to improve fertility strategies.

Histological changes in the maternal endometrium during the estrus cycle and early pregnancy are primarily influenced by the ovarian steroid hormones, estradiol and progesterone, which induce endometrial cell proliferation and differentiation (Xiao and Goff, 1999). Variations in ovarian steroid hormone production trigger the expression of growth factors and cytokines from the endometrium, subsequently affecting uterine receptivity (Spencer and Hansen, 2015). Progesterone plays a crucial role in blastocyst development by stimulating endometrial function and guiding the maternal endometrium to express genes that enhance uterine receptivity, thereby supporting the developing conceptus (Filant and Spencer, 2014). The absence of progesterone results in the animal returning to estrus due to an insufficiently developed conceptus (Spencer *et al*., 2016). During early gestation, ruminant uteri are exposed to estrogen, progesterone, and interferon-tau, leading to the initiation of endometrial gland proliferation, differentiation into secretory cells, and maintaining the secretory functions along the critical periods (Spencer and Bazer, 2002). Notably, progesterone induces endometrial transcription and downregulates progesterone receptors in luminal and glandular endometrial epithelial cells, subsequently allowing expression of genes, secretion of proteins, and function of active transports required for conceptus survival and attachment (Spencer and Hansen, 2015; Sanchez *et al*., 2018).

The appearance of cell adhesion molecules and the reduction or loss of the anti-adhesive molecules on the uterine endometrium surface are required to attain uterine receptivity (Bazer *et al*., 2011a), as shown in Table 2. Mucin (MUC1), a transmembrane glycoprotein functioning as an anti-adhesive component, is localized to the apical site of luminal endometrial epithelium but is typically reduced during the receptive and attachment phases (Carson *et al*., 2006). Estrogen stimulates MUC1 expression, while progesterone antagonizes the stimulatory actions of estrogen (Bowen *et al*., 1997). Loss of MUC1 is a prerequisite and essential for establishing uterine receptivity during the peri-implantation period. Subsequently, unmasking adhesive molecules on the surface of luminal endometrial epithelium permits initial contact with the trophectoderm of developing conceptus. Specific carbohydrate ligands, including selectins, galectins, heparin sulfate proteoglycan, cadherins, and CD44, play crucial roles in this process (Nagaoka *et al*., 2000; Illera *et al*., 2004). Low-affinity interactions are followed by stable adhesion involving integrins expressed on conceptus and luminal endometrial epithelium and their extracellular matrix bridging ligands. Integrin, a transmembrane cell adhesion protein, is a principal receptor protein with significant roles in binding and responding to the extracellular matrix (ECM), facilitating stable adhesion to components such as osteopontin and fibronectin. Moreover, integrins are pivotal in cell invasion, migration, bidirectional signaling, and cytoskeletal organization during implantation (Lessey and Arnold, 1998). According to Campbell and Humphries (2011), vertebrates possess 18 α subunits and 8 β subunits, which combine to generate approximately 24 different integrins, each characterized by distinct binding properties and tissue distributions (Campbell and Humphries, 2011). Specifically, the αvβ3 integrin has been identified in the endometrium and embryo during implantation in goats (García *et al*., 2004), sheep (Wan *et al*., 2011), and cattle (Kimmins *et al*., 2003). However, previous research indicated that in ruminants, unlike in other species, αvβ3 integrin and osteopontin do not synergistically promote embryo attachment (Kimmins *et al*., 2004). The diversity in functional integrin molecules and their expression patterns among species suggests that, despite common integrins, species-specific differences may arise due to variations in expression profiles among ruminant species (Bazer *et al*., 2011b). Elevation of integrin expression during the peri-implantation period is necessary for subsequent attachment. It regulates several genes generating cytoplasmic signaling via the mitogen-activated protein kinase pathway, activating genes pivotal to invasion processes such as matrix metalloproteinase (Nagaoka *et al*., 2000).

Moreover, a series of studies have shown that E-cadherin is required for embryo development of normal buffalo embryos and primarily influences the adhesion differentiation of the conceptus epithelium (Watson and Barcroft, 2001; Kan *et al*., 2007). The vitrification process also altered the adhesion-related gene expression in vitrified buffalo blastocyst concurrently with a low pregnancy rate (Moussa *et al*., 2019). Additionally, the study of E-cadherin localization in the oviduct and uterus of swamp buffalo during the estrous cycle suggested the dynamic and spatial change in each phase of the estrous cycle is associated with the preparation of maternal endometrium for embryo attachment (Tienthai, 2018).

However, a more comprehensive range of cellular and molecular mechanisms should be studied if they have roles in the implantation process. These aspects of implantation will elucidate the implications of implantation-related factors and the complex interactions between the conceptus and maternal endometrium that may determine the underlying mechanisms of their expression during the attachment stage. Understanding pivotal gene expression and signaling pathways that regulate endometrial functions for implantation can be used to investigate the etiology of recurrent pregnancy loss.

Table 1 Comparison of the timing of embryonic development among ruminant species.

The Stage of Development	Species		
	Ewe (day)	Cow (day)	Buffalo (day)
2-cell	1	1	ND
4-cell	1-2	2	ND
8-cell	2	3-4	4
		(Entry of embryos into the uterus)	
Morula	3-4	5-7	5
Blastocyst	4-10	7-12	5-6
Hatching Blastocyst	7-8	9-11	6-7
Elongation	11-15	12-16	13
Maternal Recognition	13-15	15-17	14-16
Pre-attachment Stage	15-18	18-22	17-24

Table 2 Cell adhesion molecules implicated in maternal-embryo interaction.

Group	Function	Member	References
Cadherins	Essential in the formation of adherens junction Assisting in properly positioning cells Necessary to allow the migration of cells Maintaining cell and tissue structure	E-cadherin β-catenin	(Coutifaris *et al.*, 1991; Poncelet *et al.*, 2002)
Mucins	Controlling the accessibility of trophoblast receptors to their ligand Downregulated in order to initiate implantation cascade Locally reduced at implantation area by blastocyst	Mucin1	(Carson *et al.*, 2000; Johnson *et al.*, 2001)
Immunoglobulin superfamily	Acting as homophilic binding and as ligands for heterophilic cell-cell adhesion Moderating trans-endothelial leukocyte migration Mediating immunological function	ICAM1 CCAM NCAM	(Svalander *et al.*, 1987)
Integrins	Interacting with extracellular matrix to conduct cellular signals between endometrial epithelial cells and conceptus Response to cytoskeletal organization, stabilize adhesion, cell migration Altered expression is correlated with infertility cases	Integrin α Integrin β	(Lessey, 1994; Burghardt *et al.*, 2002)
Carbohydrate binding receptor	Regulating trophectoderm epithelial attachment Play a role in the integrity of the epithelial sheet Mediating immunomodulation, cell adhesion, chemotaxis	CD44 Selectin Galectin	(Cichy and Pure, 2003; Jones and Aplin, 2009)

In vitro endometrial cell culture system

An *in vitro* cell culture system is a critical reproductive technology in ruminant breeding and husbandry. This system has facilitated the examination of cellular function and reproductive physiology using live animal cells for scientific purposes. Moreover, improving *in vitro* cell culture systems enhances the production of advanced embryos, which are subsequently used in *in vitro* embryo production and embryo transfer techniques. An efficient culture system for *in vitro* endometrial development should serve as a valuable model for investigating molecular mechanisms underlying implantation, which are essential for endometrial differentiation and function.

Endometrial cell culture systems have been developed for several species, particularly cattle and related species. These systems have been utilized in various studies, including those on endometrial epithelial and stromal cell function (Horn *et al.*, 1998; Lapointe *et al.*, 2000), defense mechanisms (Swangchan-Uthai *et al.*, 2012; Turner *et al.*, 2014), cell immortalization (Bai *et al.*, 2014). Findings from these culture systems have provided a better understanding of the molecular mechanisms involved in reproductive physiology and have contributed to improving strategies for ARTs.

In domestic buffalo, *in vitro* cultured endometrial epithelial cell systems have been employed as novel models to understand the maternal embryo cross talk. It has been reported that progesterone is essential for modulating the expression of cellular adhesion molecules and the related gene profiles of bubaline endometrial epithelial cells and blastocysts (Pandey *et al.*, 2021). Progesterone also influences the secretion of histotrophs in the uterine lumen, which prepares for embryonic attachment and maintains an adequate uterine environment during early embryo survival and development (Campanile and Neglia, 2007; Shah *et al.*, 2019). The endometrial cell culture system was utilized to investigate regulating the expression of genes involved in cell proliferation in the initial implantation step *(Dubey et al.*, 2023; Pal *et al.*, 2023). Currently, no studies have established an immortalized endometrial cell culture in swamp buffalo. Such a model is suggested to be pivotal for advancing the understanding of reproductive physiology in this species.

Furthermore, proteomic approaches have revealed several essential proteins associated with implantation and uterine receptivity, such as integrins, annexins, cytoskeletal proteins, and extracellular matrix proteins (Jamwal *et al.*, 2024). The proteomic profile of the bubaline endometrial epithelial cells was identified to play a crucial role in endometrial receptivity and the embryo implantation process. During the implantation receptivity phase, integrins engage with the ECM by triggering signal transduction within the cytosol through interactions with signaling molecules and cytoskeletal proteins (MacIntyre *et al.*, 2002). However, studies on the co-culture of endometrial epithelial cells and conceptuses in buffalo are still limited and much needed in this species.

These advancements enable the study of uterine receptivity physiology in Thai swamp buffaloes, particularly concerning maternal endometrium and pregnancy-related signaling. However, it is essential to note that *in vitro* cell culture systems and *in vitro* production are still less developmentally competent than their *in vivo* counterparts. There remain areas for further improvement of *in vitro* co-culture systems, which are essential for researching the attachment stage in ruminant species and developing valuable models that provide significant insights into these fields of study.

Conclusion

Swamp buffaloes are vital to the livelihoods and agricultural practices of Southeast Asia, yet they encounter significant reproductive challenges that impact their population dynamics and agricultural productivity. This review has elucidated the similarities and differences between swamp buffaloes and other ruminants, highlighting the unique aspects of buffalo reproductive physiology and the unique aspects of buffalo reproduction. Despite the adoption of ARTs, their efficacy remains below optimal levels. This emphasizes the imperative for a targeted investigation into the cellular and molecular mechanisms regulating implantation and uterine receptivity. Future research should enhance our understanding of these processes to develop more effective fertility strategies.

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