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State-of-the-art and Emerging Technologies for In Vitro Embryo Production in Buffaloes

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ABSTRACT

Buffalo is a multipurpose and economically important animal due to the demand for its products (milk and meat). Thus, the use of reproductive biotechnologies is important to maximize the diffusion of genetically superior dams and sires. After the unsatisfactory results of the Multiple Ovulation and Embryo Transfer, the combined effect of ovum pick-up from live animals and in vitro embryo production (IVEP) has great potential to dissemination of selected genetics in buffalo herds, contributing to an increase in meat and milk production. During the past two decades, considerable advances have been made in IVEP following continuous scientific effort, but at the moment their cost is not satisfactory for commercial purpose. This technique is refined day by day in order to improve the buffalo embryo quality. Thus, the objective of this paper was to review the state-of-art in IVEP, as well as discussed the emerging technologies that can contribute to improving the results of this technology in buffalo species.

Introduction

The domestic water buffalo (*Bubalus bubalis*) contributes a significant proportion of global milk production and is kept mostly by small-scale producers. Water buffaloes are classified into two subspecies: the river buffalo and the swamp buffalo. River buffaloes constitute approximately 70% of the world water buffalo population; swamp buffaloes are smaller and have lower milk yields. The world buffalo population is about 200 million, of which 97.04 % are in Asia, 2.10 % in Africa, 0.64 % in South America, 0.21 % in Europe, and 0.0001 % in Oceania (Mingala *et al*., 2017).

Buffaloes are short-day polyestrous animals (Vale and Ribeiro, 2005; Vale, 2007); indeed, in the tropical areas near the equator line they are polyestrous (Perera, 2011). However, they have relatively poor reproductive efficiency throughout the world, irrespective of their location. They exhibit many of the known reproductive disorders, including delayed onset of puberty, poor estrus expression, longer postpartum ovarian quiescence, and most importantly, lowered conception rates, particularly when bred artificially (Ponraj *et al*., 2017).

Several reproductive biotechniques are used to minimize or even solve reproductive failures in different domestic species, including buffaloes. Among these biotechniques, it is important to mention estrus synchronization (Rao and Rao, 1983), artificial insemination (Vale, 1994), and multiple ovulation and embryo transfer (MOET), which is the in vivo production of embryos for later transfer to previously prepared recipients (Techakumph *et al*., 2001).

The in vitro embryo production (IVEP) is being used in buffaloes as another reproductive tool for the genetic improvement of the herd and to increase the productive indices (Fig. 1). Embryos produced by in vitro techniques have been used, resulting in pregnancies and calf births in buffalo (Galli *et al*., 2001), but the success rate in terms of yield of transferable-quality embryos and number of calf births has been low (Nandi *et al*., 2002).

Recently viewed, emerging "omics" technologies have been used in livestock production. Also, these technologies give new tools for improving reproduction in buffalo. Thus, this review summarized the different steps involved in IVEP in buffalo, analyzed the problems associated with this technique, and presented new approaches aimed at improving this biotechnique.

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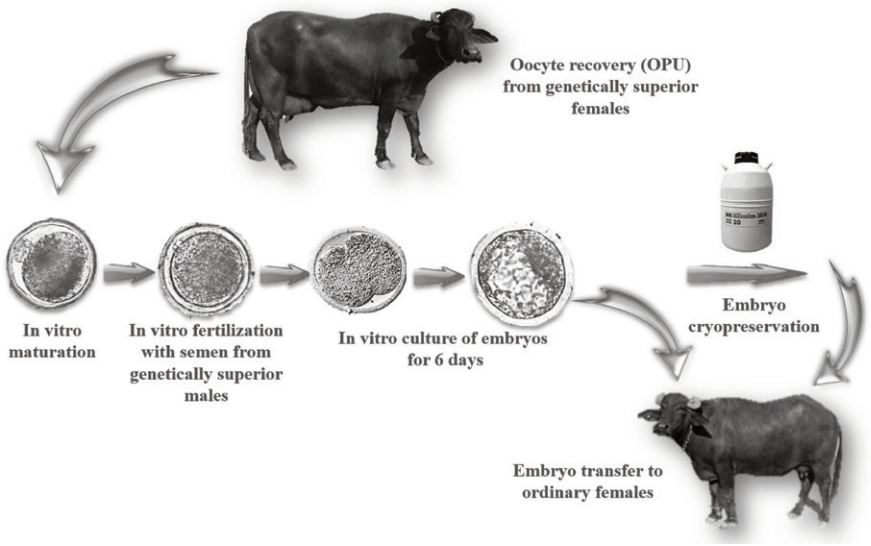

Fig. 1. Scheme of a genetic improvement program in buffalo based on the use of in vitro embryo production.

Oocyte recovery

Oocytes recovery method is an important step during IVEP because oocytes quality directly affects the quality and quantity of the embryos produced (Nandi *et al.*, 2002). Although abattoir-derived ovaries provide a cheap and abundant source of oocytes (Das *et al.*, 1996), oocyte recovery from live animals is the preferred method in buffaloes for commercial purpose. Therefore, transvaginal ovum pick-up (TVOPU) and laparoscopic ovum pick-up (LOPU) will be discussed, as they are of interest to the industry of buffalo embryo production.

TVOPU is usually performed in centers where the environment can be controlled for ease of working. Various scanners and probes are suitable, but generally, a 5-6.5 MHz vaginal probe is used in buffalo. The puncture needle varies from 17 to 20G and is connected to a vacuum pump regulated from 40 to 115 mmHg (Manjunatha *et al.*, 2008). The follicular aspirate is collected in a tube, which is kept warm in a heating block (Galli *et al.*, 2014). The combination of TVOPU with IVEP provides an alternative approach for the superior genetic exploitation and multiplication (Boni *et al.*, 1996). The hormonal stimulation with follicle stimulating hormone (FSH) prior to TVOPU has been successfully used in IVEP programs in cattle, resulting in the increase of total embryos produced per session (Vieira *et al.*, 2014). In buffalo, the stimulation with FSH increased the proportion of large and medium-sized follicles available and resulted in greater blastocyst rates and embryo yield per TVOPU session (Carvalho *et al.*, 2019).

LOPU is the procedure of choice for the recovery of oocytes from species and categories where TVOPU is difficult or impossible, such as small ruminants (Cognié *et al.*, 2004), prepubertal bovines and buffaloes (Baldassarre and Bordignon, 2018) and wild animals (Locatelli *et al.*, 2006). This procedure is a very safe and minimally invasive technique with no intra-operative complications, which also does not affect the future reproductive efficiency of females (Baldassarre and Bordignon, 2018). In another study comparing three groups of age (< 120 days, 120-150 days and > 150 days) of prepubertal female buffalo calves, the mean recovery rate was 85.7% and was not observed significant difference among groups of age for the number of recovered oocytes (Baldassarre *et al.*, 2017).

In cattle, there have been an increasing interest in studies concerning antral follicle count and its influence on the reproductive performance, as well as its applications in reproductive biotechnologies (Rico *et al.*, 2012). Thus, Ohashi *et al.* (2017) used as oocyte donors only buffaloes that had, on ultrasound examination, more than 10 antral follicles per ovary. The number of aspirated follicles, recovered oocytes and blastocyst rate were above the average in buffaloes, demonstrating that the selection of oocyte donors can contribute to increase the number of viable oocytes and thereby improve IVEP rates and decrease the pregnancy cost. The aspirated follicles and oocyte recovery rates by TVOPU in different studies are presented in Table 1.

In vitro maturation (IVM) of oocytes

IVM is a critical step and makes the immature oocytes competent for in vitro fertilization (IVF). It is characterized by the resumption of meiosis, nucleus morphology changes, perivitelline space growth and expansion of cumulus cells (Mondadori *et al.*, 2010). The IVM rate in buffalo is approximately 80%, similar to that obtained in bovine (Santos *et al.*, 2002; Hammam *et al.*, 2010). However, the maturation rate observed in females aged 2 to 6 months are much lower (40-50%) from those obtained in adult animals (Baldassarre *et al.*, 2017).

Basically, the medium and culture conditions are the same as those used for bovine oocytes (Galli *et al.*, 2001). The culture medium, protein supplements and hormones play an important role in the maturation rate and embryonic development following IVF (Mahmoud and El-Naby, 2013). Different culture media have been used for IVM of buffalo oocytes, such as Ham's F-10 (Totey *et al.*, 1993b), minimum essential medium – MEM (Ravindranatha *et al.*, 2001), and TCM199 (Pandey *et al.*, 2010). However, TCM199 gives higher maturation rates, which may be related to factors in its composition, such as essential amino acids and glutamine that stimulate DNA and RNA synthesis and enhance cell division (Gordon, 2003).

The time required for nuclear maturation of oocytes in vitro, which means the arrival to metaphase II (MII) stage, is from 18 to 24 h (Gasparrini *et al.*, 2008). Moreover, the increase in the duration of IVM is related to a decrease in the blastocyst rates (Oba and Camargos, 2011). Thus, maturation periods longer than 24 h may lead to inappropriate chromatin configurations, oocyte aging and a decrease in oocyte competence to development (Kumar and Anand, 2012).

A study has shown that fetal calf serum contains fetuin, a

Table 1. Oocyte recovery rates by transvaginal ovum pick up and subsequent cleavage/blastocyst rates obtained by in vitro embryo production in buffaloes

Aspirated follicles[1]	Recovered oocytes1	%	Aspiration frequency	Cleavage rate (%)	Blastocyst rate (%)	Blastocyst/ buffalo/session	References
6.8 ± 0.3	4.1 ± 0.5	57.7	Twice a week (control)	41.7	26	1.2 ± 0.2	Sá Filho *et al.* (2009)
9.1 ± 0.6	5.2 ± 0.5	54.5	Twice a week (bovine GH)	46.2	19.7	1.3 ± 0.6	
4.8 ± 0.3	2.3 ± 0.1	53.6	Twice a week (mid-winter)	52.7	8.6	-	Di Francesco *et al.* (2012)
4.7 ± 0.2	2.2 ± 0.1	49.3	Twice a week (spring-summer)	59.8	6.4	-	
4.2 ± 0.2	2.2 ± 0.2	57.4	Twice a week (autumn)	65.6	23.2	-	
5.3 ± 0.2	2.7 ± 0.2	50	-	53.5	11.5	0.3 ± 0.1	Gasparrini *et al.* (2014)
14.8 ± 0.8	9.9 ± 0.6	73.6[a]	14-day interval	32.7	19.5	1.7 ± 0.4	Ferraz *et al.* (2015)
11.3 ± 0.5	7.6 ± 0.4	69.3[a]	7-day interval	33.4	18.6	1.3 ± 0.2	
17.3 ± 1.0	10.1 ± 0.7	58.5[b]	14-day interval + bovine GH	26	13.4	0.8 ± 0.2	
14.3 ± 0.6	9.3 ± 0.4	67.4[a]	7-day interval + bovine GH	35.1	9.6	0.7 ± 0.1	
273 (total)	4.5 ± 0.5[a]	51.0[a]	14-day interval	64.0[a]	28.0[a]	-	Konrad *et al.* (2017)
266 (total)	2.8 ± 0.5[b]	31.5[b]	7-day interval	44.0[a]	6.0[b]	-	
13.5 ± 5.6	10.2 ± 6.5	76	7-day interval	-	21.8[A] 23.0[B] 17.0[C]		Marin *et al.* (2019a)

[1]Buffalo/session; [a,b] $P < 0.05$; [A,B,C] Bull 1, 2 and 3, respectively.

major glycoprotein that can prevent hardening of the zona pellucida during IVM, improves the fertilization capacity of oocytes (Schroeder *et al.*, 1990). The developmental competence of in vitro matured oocytes has been improved by addition of hormones such as follicle stimulating hormone - FSH (Hegab *et al.*, 2009), equine chorionic gonadotrophin (Gupta *et al.*, 2001), luteinizing hormone - LH and estradiol to culture media (Nandi *et al.*, 2002).

During heat stress, buffalo yield fewer good quality oocytes than under unstressed conditions. High ambient temperature and humidity have a deleterious effect on oocyte capability to mature in vitro (Zoheir *et al.*, 2007). However, Nandi *et al.* (2001) stated that external environmental conditions did not affect fertilization if the aspirated oocytes successfully completed maturation.

The relationship between follicle size from which oocytes were aspirated and IVM in buffalo was studied by Yousaf and Chohan (2003). The authors found poor IVM rates (32% vs 32.7%) for oocytes isolated from 2-3 mm and from 3-4 mm follicles respectively, whereas significantly more oocytes reached maturation from 4-6 mm than 6-8 mm follicles (67.1% vs 79.1%, respectively).

Mahmoud *et al.* (2010) have demonstrated that the presence of cumulus cells is necessary for cytoplasmic and/or nuclear maturation. It has also been reported that cumulus cell presence prevents hardening of the zona pellucida, provides energy for oocyte maturation, produces cytoplasmic maturation factors, and helps in fertilization (Tanghe *et al.*, 2002).

Santos *et al.* (2002) have observed that the MII started after 18 h of maturation but that most oocytes completed nuclear maturation between 21 and 24 h. In contrast, Neglia *et al.* (2001) found that most buffalo oocytes reached the MII stage between 15 h and 19 h after the start of IVM and an increased incidence of degenerated oocytes was observed at later times.

IVF of matured oocytes

In buffalo, fresh and frozen semen can be used for IVF. The frozen semen is more desirable because it ensures homogeneity in the experimental data, but mainly because it is the most commercially indicated. The cleavage rates (~45-50%) in buffaloes (Suresh *et al.*, 2009) after IVF are lower as compared to those obtained in bovine (~70%) (Sales *et al.*, 2015). In buffalo, studies revealed that frozen semen used from the different bulls has been the source of variation for IVF (Shi *et al.*, 1990). However, Marin *et al.* (2019a) did not observe any significant difference by using frozen semen from two different buffalo bulls.

The protocols for the preparation of semen, including washing and capacitation for IVF, are basically the same as those used for bovine (Parrish, 2014). Most of the described protocols have used heparin for in vitro sperm capacitation (Totey *et al.*, 1993a). However, alternative substances such as nitric oxide donors (Jagan Mohanarao and Atreja, 2012), methyl-β-cyclodextrin (Elkhawagah *et al.*, 2014), or osteopontin (Boccia *et al.*, 2013) have shown potentially useful results.

The incubation time of sperm cells with oocytes play a significant role; it should be adequate to enable correct induction of plasma membrane vesiculation and the complete acrosomal reaction. A minimum time for co-incubation of 4-6 h has been proposed. However, it was reported that this time should be extended by at least 16 h to maximize blastocyst production rates (Kumar and Anand, 2012). It was established that if the co-cultivation time is greater than 20 h, increased incidence of polyspermy could result, eventually reducing the blastocyst production rates (Gasparrini *et al.*, 2008). In addition to co-cultivation time, sperm concentration should be adequate to obtain better results during IVF (Marin *et al.*, 2019b). The cleavage rates after IVF from different studies are presented in Table 1.

In vitro culture (IVC) of embryos

Different culture media have been tested for IVC of buffalo embryos, such as culture in sheep oviduct (Galli *et al.*, 2001), somatic cell co-culture systems (Dantas, 2002), culture media supplemented with blood serum or semi-defined media supplemented with bovine serum albumin (Wadhwa *et al.*, 2009), and recently, synthetic oviductal fluid medium supplemented with 5% fetal bovine serum (Marin *et al.*, 2019a). However, irrespective of the media used, blastocyst formation rates are usually lower in buffaloes, around 22% (Suresh *et al.*, 2009; Baruselli *et al.*, 2018), as compared to cattle (~ 40%) (Sales *et al.*, 2015). The blastocyst rates in IVEP studies are presented in Table 1.

There is no adequate information about the metabolic and biochemical needs of buffalo embryos, which has made it difficult to develop suitable culture media for this species. The observed improvements in the blastocyst production rates could be mainly due to changes in the IVM and IVF systems, rather than changes tested during the IVC period (Gasparrini,

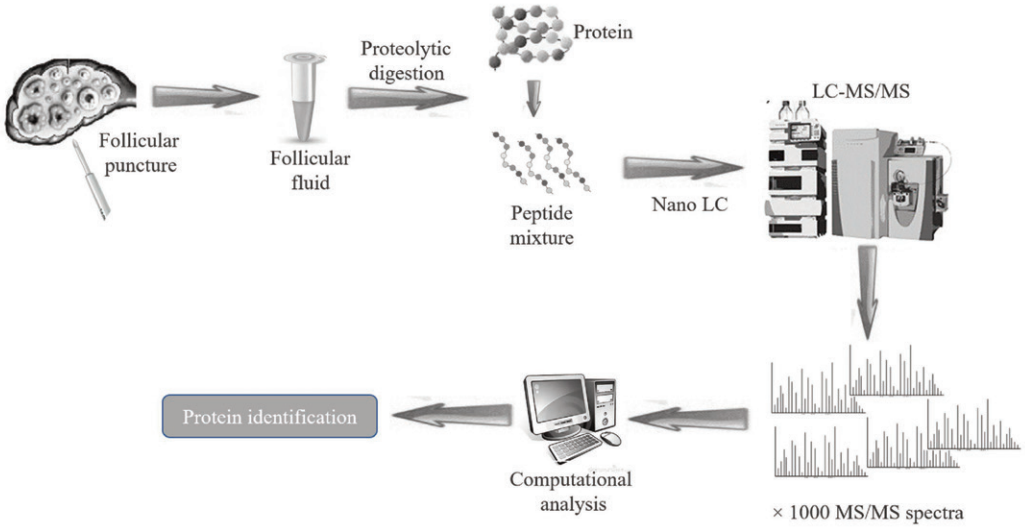

Fig. 2. Shotgun proteomics analysis of fluid collected by follicular puncture: denaturation of extracted proteins is followed by proteolytic digestion of proteins into peptides. These peptides are fractionated by liquid chromatography (LC) and identified after tandem mass spectrometry (MS/MS). The proteins are identified after protein database search and computational analysis (Adapted from Langley *et al*., 2013).

2013). In addition, it was observed that in in vitro or in vivo conditions, buffalo embryos develop 12 to 24 h faster than bovine embryos, indicating that the metabolism has specific characteristics (Galli *et al*., 2001).

Addition of glucose to the culture medium has been reported to have an important role in oocyte and embryo culture in many species (Preis *et al*., 2005). In buffalo, studies have shown that embryos require adequate glucose concentration for their proper development from the earliest culture stages (Suárez Novoa *et al*., 2011). Gasparrini (2013) observed that during the early development of buffalo embryos around the fourth day of culture, they required relatively high glucose concentrations (1.5 mM), while a decreased concentration or even absence of glucose in the late culture stages did not show any deleterious effects. In addition, Kumar and Anand (2012) observed promising results during oocyte maturation and embryo culture when the culture media was supplemented with high glucose concentrations (5.6 mM).

Emerging technologies

In the field of assisted reproduction technologies (ART), technical advances have progressed at a much faster pace than the understanding of the underlying physiology. In the buffalo, as for other farm animals, the potential impacts of the vast array on IVEP have not been thoroughly assessed. In this review, will be specifically addressed: transcriptomics and proteomics.

Transcriptomics

The set of transcripts, or transcriptome, can help to decode the information on cell metabolism and functioning (Wang *et al*., 2009). The transcriptome is the whole set of transcripts in the cell and can be generated using hybridization (microarray) and Next Generation Sequencing of RNA (RNA-seq). Gene expression studies were done with microarrays. However, issues with microarrays include cross-hybridization, poor quantification of lowly and highly expressed genes, and needing to know the sequence. Thus, transcriptomics transitioned to sequencing-based methods (Kukurba and Montgomery, 2015).

The use of RNA-seq to identify molecular markers of quality of oocyte, sperm and embryo has already been used in farm animals such as bovine (Gilchrist *et al*., 2016), ovine (Wu *et al*., 2017) and porcine (Cao *et al*., 2014). Li *et al*. (2018) used RNA-seq of follicular granulosa cells to identify genes which may affect fertility in buffalo. Forty suggestive loci (related to 28 genes) were identified to be associated with six reproductive traits (first, second and third calving age, calving interval, the number of services per conception and open days).

RNA-seq can provide a better understanding the in vitro culture influence on embryo quality. For example, an RNA-seq study compared the transcriptome profile of bovine embryos produced in either FBS-containing or FBS-free media to the profile of in vivo blastocysts (Heras *et al*., 2016). It was observed that FBS-free embryos were more similar to in vivo embryos. However, after looking at the lipid and amino acid pathway gene expression profiles, it was concluded that FBS-free and in vivo-derived embryos were still very different. Following the reports of biomarker genes, then future challenge will be correlations with embryo development and pregnancy rates.

Proteomics

Proteomics is the analysis of the entire protein complement of a cell, tissue, or organism under a specific, defined set of conditions. In its present state, it is dependent on decades of technological and instrumental developments. These developments have included advances in mass spectrometry (MS) technology, protein fractionation techniques, bioinformatics, etc.

The mechanism regulating embryo development under reduced oxygen tension remains elusive. Thus, Shahzad *et al*. (2020) cultured buffalo embryos under 5% or 20% oxygen and used iTRAQ-based quantitative proteomics. Functional analysis indicated that 43 differentially expressed proteins were associated with glycolysis and fatty acid degradation. The data suggested that higher lipid degradation, an elevated cholesterol level and a higher unsaturated to saturated fatty acid ratio might be involved in the better cryo-survival ability reported in embryos cultured under low oxygen.

Insights into the follicular fluid composition provide a useful indication of the requirements for in vitro oocyte maturation and may be used as a guide for the inexpensive

formulation of cell culture conditions. Recently, limited data have been obtained on the fractionated follicular fluid proteins and their effects on in vitro embryo development. Thus, proteomic profile has already been studied in several species, including bovine (Ferrazza *et al*., 2017), goats (Paula Junior *et al*., 2018) and deer (Souza *et al*., 2020).

For the studies on follicular fluid proteomics, the most indicated technique is the "shotgun proteomics" which eliminates the need for extracting proteins from gels and was introduced to identify and/or quantify the maximal number of proteins in a given sample in a high throughput and robust manner (Fig. 2). Thus, Fu *et al*. (2016) used a proteomic-based approach to analyze the proteome of buffalo follicular fluid. In total, 363 proteins were identified and classified. Additionally, to evaluate difference in proteins expressed between buffalo follicular fluid with different follicle size (small and large), a quantitative proteomic analysis based on multi-dimensional liquid chromatography pre-fractionation tandem Orbitrap mass spectrometry identification was performed. Eleven differentially expressed proteins (six downregulated and five upregulated in large follicles) were identified and assigned to a variety of functional processes, including serine protease inhibition, oxidation protection and the complement cascade system. These results offer new information about proteins present in buffalo follicular fluid and should facilitate the development of new biomarkers.

To investigate the protein profiling of buffalo oocytes at the germinal vesicle stage and MII stage, an iTRAQ-based strategy was applied by Chen *et al*. (2016). A total of 3,763 proteins were identified. Among these proteins identified, 173 proteins were differentially expressed in germinal vesicle oocytes and competent MII oocytes, and 146 proteins were differentially abundant in competent and incompetent matured oocytes.

Conclusion

The IVEP in buffalo are steadily increasing due to the demand for increasing the productivity using elite animals. However, this technique is challenged by many factors, such as infrastructure and low success rates when compared to bovine. The use of emerging technologies (transcriptomics and proteomics) will provide valuable information for understanding the molecular mechanism underlying oocyte maturation and embryo development. In addition, the results provided using these technologies may potentially act as markers to predict developmental competence during IVEP in buffalo.

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Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

References

Baldassarre, H., Bordignon, V., 2018. LOPU in calves for in vitro embryo production. Animal Reproduction 15, 191-196.

Baldassarre, H., Littkemann, M., Currin, L., Macedo, M.P., Gutierrez, K., Bordignon, V., 2017. Efficient shortening of generation interval by applying laparoscopic ovum pickup and in vitro embryo production technologies in Mediterranean buffalo of 2-6 months of age. In: Proceedings of the IX Symposium of the International Buffalo Federation. Campeche, Mexico, pp. 231.

Baruselli, P.S., Soares, J.G., Bayeux, B.M., Silva, J.C.B., Mingoti, R.D., Carvalho, N.A.T., 2018. Assisted reproductive technologies (ART) in water buffaloes. Animal Reproduction 15, 971-983.

Boccia, L., Di Francesco, S., Neglia, G., De Blasi, M., Longobardi, V., Campanile, G., 2013. Osteopontin improves sperm capacitation and in vitro fertilization efficiency in buffalo (*Bubalus bubalis*). Theriogenology 80, 212-217.

Boni, R., Roviello, S., Zicarelli, L., 1996. Repeated ovum pick-up in Italian Mediterranean buffalo cows. Theriogenology 46, 899-909.

Cao, S., Han, J., Wu, J., Li, Q., Liu, S., Zhang, W., Pei, Y., Ruan, X., Liu, Z., Wang, X., Lim, B., Ning, L., 2014. Specific gene-regulation networks during the preimplantation development of the pig embryo as revealed by deep sequencing. BMC Genomics 15, 4. o *et al*. BMC Genomics 2014, 15:4.

Carvalho, J.G.S., Carvalho, N.A.T., Bayeux, B.M., Watanabe, Y.F., Watanabe, O.Y., Mingoti, R.D., Baruselli, P.S., 2019. Superstimulation prior to the ovum pick-up improves the in vitro embryo production in nulliparous, primiparous and multiparous buffalo (*Bubalus bubalis*) donors. Theriogenology 138, 164-168.

Chen, L., Zhai, L., Qu, C., Zhang, C., Li, S., Wu, F., Qi, Y., Lu, F., Xu, P., Li, X., Shi, D., 2016. Comparative proteomic analysis of buffalo oocytes matured in vitro using iTRAQ technique. Scientific Reports 6, 31795.

Cognié, Y., Poulin, N., Locatelli, Y., Mermillod, P., 2004. State-of-the-art production, conservation and transfer of in-vitro-produced embryos in small ruminants. Reproduction Fertility and Development 16, 437-445.

Dantas, J.K., 2002. Desenvolvimento de embriões bubalinos (*Bubalus bubalis*) cultivados in vitro em diferentes meios. PhD thesis, Universidade Federal do Pará, 2002.

Das, G.K., Jain, G.C., Solanki, V.S., Tripathi, V.N., 1996. Efficacy of various collection methods for oocyte retrieval in buffalo. Theriogenology 46, 1403-1411.

Di Francesco, S., Novoa, M.V., Vecchio, D., Neglia, G., Boccia, L., Campanille, G., Zicarelli, L., Gasparrini, B., 2012. Ovum pick-up and in vitro embryo production (OPU-IVEP) in Mediterranean Italian buffalo performed in different seasons. Theriogenology 77,148-154.

Elkhawagah, A.R., Longobardi, V., Gasparrini, B., Sosa, G.A., Salzano, A., Aboul-roos, M.E.A., Abd El-Gaffar, A.E., Zicarelli L., 2014. Effect of Methyl-B-Cyclodextrin (MBCD) on in vitro capacitation of buffalo frozen/thawed sperm. Journal of Buffalo Science 3, 12-17.

Ferraz, M.L., Sá Filho, M.F., Batista, E.O.S., Watanabe, Y.F., Watanabe, M.R., Dayan, A., 2015. Paradoxical effects of bovine somatotropin treatment on the ovarian follicular population and in vitro embryo production of lactating buffalo donors submitted to ovum pick-up. Animal Reproduction Science 154, 1-7.

Ferrazza, R.A., Garcia, H.D.M., Schmidt, E.M.D.S., Mihm Carmichael, M., Souza, F.F., Burchmore, R., Sartori, R., Eckersall, P.D., Ferreira, J.C.P., 2017. Quantitative proteomic profiling of bovine follicular fluid during follicle development. Biology of Reproduction 97, 835-849.

Fu, Q., Huang, Y., Wang, Z., Chen, F., Huang, D., Lu, Y., Liang, X., Zhang, M., 2016. Proteome profile and quantitative proteomic analysis of buffalo (*Bubalus bubalis*) follicular fluid during follicle development. International Journal of Molecular Science 17, 618-638.

Galli, C., Crotti, G., Notari, C., Turini, P., Duchi, R., Lazzari, G., 2001. Embryo production by ovum pick up from live donors. Theriogenology 55, 1341-1357.

Galli, C., Duchi, R., Colleoni, S., Lagutina, I., Lazzari, G., 2014. Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice. Theriogenology 81, 138-151.

Gasparrini, B., 2013. In vitro embryo production in buffalo: yesterday, today and tomorrow. Buffalo Bulletin 32, 188-195.

Gasparrini, B., De Rosa, A., Attanasio, L., Boccia, L., Di Palo, R., Campanile, G., Zicarelli, L., 2008. Influence of the duration of in vitro maturation and gamete co-incubation on the efficiency of in vitro embryo development in Italian Mediterranean buffalo (*Bubalus bubalis*). Animal Reproduction Science 105, 354-364.

Gasparrini, B., Neglia, G., Di Palo, R., Di Vecchio, D., Albero, G., Esposito, L., Zicarelli, L., 2014. Influence of oocyte donor on in vitro embryo production in buffalo. Animal Reproduction Science 144, 95-101.

Gilchrist, G.C., Tscherner, A., Nalpathamkalam, T., Merico, D., LaMarre, J., 2016. MicroRNA expression during bovine oocyte maturation and fertilization. International Journal of Molecular Science 17, 396.

Gordon, I. 2003. Laboratory Production of Cattle Embryos. Second Ed. CABI Publishing, Wallingford, UK, pp. 112-150.

Gupta, P.S., Nandi, S., Ravindranatha, B.M., Sarma, P.V., 2001. Effect of commercially available PMSG on maturation, fertilization and embryo development of buffalo oocytes in vitro. Reproduction Fertility and Development 13, 355-360.

Hammam, A.M., Whisnant, C.S., Elias, A., Zaabel, S.M., Hegab, A.O., Abu-El-Naga, E.M., 2010. Effect of media, sera and hormones on in vitro maturation and fertilization of water buffalos (*Bubalus bubalis*). Journal of Animal and Veterinary Advances 9, 27-31.

Hegab, A.O., Montasser, A.E., Hammam, A.M., Abu El-Naga, E.M.A., Zaabel, S.M., 2009. Improving in vitro maturation and cleavage rates of buffalo oocytes. Animal Reproduction 6, 416-421.

Heras, S., De Coninck, D.I.M., Van Poucke, M., Goossens, K., Bogado, P.O., Van Nieuwerburgh, F., Deforce, D., De Sutter, P., Leroy, J.L.M.R., Gutierrez-Adan, A., Peelman, L., Van Soom, A., 2016. Suboptimal culture conditions induce more deviations in gene expression in male than female bovine blastocysts. BMC Genomics 17, 72.

Jagan Mohanarao, G., Atreja, S.K., 2012. Identification of NO induced and capacitation associated tyrosine phosphoproteins in buffalo (*Bubalus bubalis*) spermatozoa. Research in Veterinary Science 93, 618-623.

Konrad, J., Clérico, G., Garrido, M.J., Taminelli, G., Yuponi, M., Yuponi, R., Sansinena, M., 2017. Ovum pick-up interval in buffalo (*Bubalus bubalis*) managed under wetland conditions in Argentina: Effect on follicular population, oocyte recovery, and in vitro embryo development. Animal Reproduction Science 183, 39-45.

Kumar, D., Anand, T., 2012. In vitro embryo production in buffalo: basic concepts. Journal of Buffalo Science 1, 50-54.

Kukurba, K.R., Montgomery, S.B., 2015. RNA sequencing and analysis". Cold Spring Harbor Protocols 2015, 951-969.

Langley, S.R., Dwyer, J., Drozdov, I., Yin, X., Mayr, M., 2013. Proteomics: from single molecules to biological pathways. Cardiovascular Research 97,612-622.

Li, J., Liu, J., Campanile, G., Plastow, G., Zhang, C., Wang, Z., Cassandro, M., Gasparrini, B., Salzano, A., Hua, G., Liang, A., Yang, L., 2018. Novel insights into the genetic basis of buffalo reproductive performance. BMC Genomics. 19, 814.

Locatelli, Y., Vallet, J.C., Huyghe, F.P., Cognié, Y., Legendre, X., Mermillod, P., 2006. Laparoscopic ovum pick-up and in vitro production of sika deer embryos: effect of season and culture conditions. Theriogenology 66, 1334-1342.

Mahmoud, K.G.M., El-Naby, A.H.H., 2013. Factors affecting buffalo oocyte maturation. Global Veterinaria 11, 497-510.

Mahmoud, K., Ghi, M., Mohamed, Y.M.A., Amer, M.A, Noshy, M.M., Nawito, M.F., 2010. Aneuploidy in in vitro matured buffalo oocytes with or without cumulus cells. Nature and Science 8, 46-51.

Manjunatha BM, Ravindra JP, Gupta PS, Devaraj M, Nandi S., 2008. Oocyte recovery by ovum pick up and embryo production in river buffaloes (*Bubalus bubalis*). Reproduction in Domestic Animals 43, 477-4780.

Marin, D.F.D., Souza, E.B., Brito, V.C., Nascimento, C.V., Ramos, A.S., Rolim Filho, S.T., Costa, N.N., Cordeiro, M.S., Santos, S.S.D., Ohashi, O.M., 2019a. In vitro embryo production in buffaloes: from the laboratory to the farm. Animal Reproduction 16, 260-266.

Marin, D.F.D., Costa, N.N., Santana, P. P.B., Souza, E.B., Ohashi, O.M., 2019b. Importance of lipid metabolism on oocyte maturation and early embryo development: can we apply what we know to buffalo? Animal Reproduction Science, 106220.

Mingala, C.N., Villanueva, M.A., Cruz, L.C., 2017. River and swamp buffaloes: history, distribution and their characteristics. In: The Buffalo (*Bubalus bubalis*): Production and Research. Bentham Science Publishers, Sharjah, UAE, pp. 3-31.

Mondadori, R.G., Santin, T.R.S., Fidelis, A.A.G., Name, K.P.O., Silva, J.S., Rumpf, R., Báo, S., 2010. Ultrastructure of in vitro oocyte maturation in buffalo (*Bubalus bubalis*). Zygote 18, 309-314.

Nandi, S., Chauhan, M.S., Palta, P., 2001. Effect of environmental temperature on quality and developmental competence in vitro of buffalo oocytes. The Veterinary Record 148, 278-279.

Nandi, S., Raghu, H.M., Ravindranatha, B.M., Chauha, M.S. 2002. Production of buffalo (*Bubalus bubalis*) embryos in vitro: premises and promises. Reproduction in Domestic Animals 37, 65-74.

Neglia, G., Marino, M., Di Palo, R., Wilding, M., Caracciolo, M., di Brienza, V., Dale, B., Gasparrini, B., Zicarelli, L.A., 2001. A comparison of in vitro maturation in buffalo (*Bubalus bubalis*) and bovine oocytes using confocal microscopy. Theriogenology 55. 488.

Oba, E., Camargos, A.S., 2011. Produção in vitro de embriões bubalinos. Revista Brasileira de Reprodução Animal 35, 80-87.

Ohashi, O.M., Almeida, N.N.C., Cordeiro, M.S., Rolim Filho, S.T., Ribeiro, H.F.L., Santos, A.X., Ayala, H.M.D., Brito, V.C., Ramos, A.S., Silva, T.V.G., Santos, S.S.D., Miranda, M.S., 2017. Produção in vitro de embrião (PIVE) na espécie bubalina. Revista Brasileira de Reprodução Animal 41, 195-200.

Pandey, A., Gupta, S.C., Singh, N., Rana, J.S., Gupta, N., 2010. Efficiency of SCNT buffalo (*Bubalus bubalis*) embryos in different culture medium and analysis of mRNA expression of insulin-like growth factors during embryogenesis. Reproduction in Domestic Animals 45, 786-795.

Parrish, J.J., 2014. Bovine in vitro fertilization: in vitro oocyte maturation and sperm capacitation with heparin. Theriogenology 81, 67-73.

Paula Junior, A.R., van Tilburg, M.F., Lobo, M.D.P., Monteiro-Moreira, A.C.O., Moreira, R.A., Melo, C.H.S., Souza-Fabjan, J.M.G., Araújo, A.A., Melo, L.M., Teixeira, D.I.A., Moura, A.A., Freitas, V.J.F., 2018. Proteomic analysis of follicular fluid from tropically-adapted goats. Animal Reproduction Science 188, 35-44.

Perera, B.M., 2011. Reproductive cycles of buffalo. Animal Reproduction Science 124, 194-199.

Ponraj, P., Chang, S., Rajesh, N.V., Veeraselvam, M., Rajesh, K.D., 2017. Prevalence of various pathological conditions in female buffaloes (*Bubalus bubalis*). Asian Pacific Journal of Reproduction 6, 58-67.

Preis, K.A., Seidel, G.E., Gardner, D.K., 2005. Metabolic markers of developmental competence for in vitro-matured mouse oocytes. Reproduction 130, 475-483.

Rao, A.R., Rao, C.C., 1983. Synchronisation of oestrus and fertility in buffaloes with a progesterone releasing intravaginal device. The Veterinary Record 113, 623-624.

Ravindranatha, B.M., Nandi, S., Gupta, S.P.S., Sharma, P.V., 2001. Comparison of three different media on maturation of buffalo oocytes in vitro. Indian Journal of Animal Science 71, 841-843.

Rico, C., Drouilhet, L., Salvetti, P., Dalbiès-Tran, R., Jarrier, P., Touzé, J-L., Pillet, E., Ponsart, C., Fabre, S., Monniaux, D., 2012. Determination of anti-Müllerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: from the laboratory to the farm. Reproduction Fertility and Development 24, 932-944.

Sá Filho, M.F., Carvalho, N.A.T., Gimenes, L.U., Torres-Júnior, J.R., Nasser, L.F.T., Tonhati, H., Garcia, J.M., Gasparrini, B., Zicarelli, L., Baruselli, P.S., 2009. Effect of recombinant bovine somatotropin (bST) on follicular population on in vitro buffalo embryo production. Animal Reproduction Science, 113, 51-59.

Sales, J.N.S., Iguma, L.T., Batista, R.I.T.P., Quintão, C.C.R., Gama, M.A.S., Freitas, C., Pereira, M.M., Camargo, L.S., Viana, J.H., Souza, J.C., Baruselli, P.S., 2015. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. Journal of Dairy Science 98, 3086-3099.

Santos, S.S.D., Costa, S.H.F., Dantas, J.K., Ohashi, O.M., 2002. Maturação in vitro de oócitos bubalinos. Revista Brasileira de Reprodução Animal 26, 37-42.

Schroeder, A., Schultz, R.M., Kopf, G.S., Taylor, E.R., Becker, R.B., Eppig, J.J., 1990. Fetuin inhibit zona pellucida hardening and conversion of ZP2 to ZP2f during spontaneous mouse oocyte maturation in the absence of serum. Biology of Reproduction 43, 891-897.

Shahzad, Q., Pu, L., Wadood, A.A., Waqas, M., Xie, L., Pareek, C.S., Xu, H., Liang, X., Lu, Y., 2020. Proteomics analysis reveals that Warburg Effect along with modification in lipid metabolism improves in vitro embryo development under low oxygen.

International Journal of Molecular Science 21,1996.
Shi, D.S., Lu, K.H., Gordon, I., 1990. Effects of bulls on fertilization of bovine oocytes and their subsequent development in vitro. Theriogenology 33, 324.
Souza, T.T.S., Bezerra, M.J.B., van Tilburg, M.F., Nagano, C.S., Rola, L.D., Duarte, J.M.B., Melo, L.M., Moura, A.A., Freitas, V.J.F., 2019. Protein profile of the ovarian follicular fluid of brown brocket deer (Mazama gouazoubira; Fisher, 1814). Zygote 28, 170-173.
Suárez Novoa, M.V., Di Francesco, S., Rubessa, M., Boccia, L., Longobardi, V., De Blasi, M., Gasparrini, B., 2011. Effect of reducing glucose concentration during in vitro embryo culture in buffalo (*Bubalus bubalis*). Reproduction Fertility and Development 23, 168.
Suresh, K.P., Nandi, S., Mondal, S., 2009. Factors affecting laboratory production of buffalo embryos: a meta-analysis. Theriogenology 72, 978-985.
Tanghe, S., Van Soom, A., Nauwynck, H., Coryn, M., De Kruif, A., 2002. Minireview: Functions of cumulus oophorus during oocyte maturation, ovulation and fertilization. Molecular Reproduction and Development 61, 414-424.
Techakumph, M., Sukavong, Y., Yienvisavakul, V., Buntaracha, B., Pharee, S., Intaramongkol, S., Apimeteetumrong, M., Intaramongkol, J., 2001. The transfer of fresh and frozen embryos in an elite swamp buffalo herd. The Journal of Veterinary Medical Science 63, 849-852.
Totey, S.M., Pawshe, C.H., Singh, G.P., 1993a. Effects of bull and heparin and sperm concentrations on in vitro fertilization of buffalo (*Bubalus bubalis*) oocytes matured in vitro. Theriogenology 39, 887-898.
Totey, S.M., Pawshe, C.H., Singh, G.P., 1993b. In vitro maturation and fertilization of buffalo oocytes (*Bubalus bubalis*): Effects of media, hormones and sera. Theriogenology 39, 1153- 1171.
Vale, W.G., 1994. Collection, processing and deep freezing of buffalo semen. Buffalo Journal 2, 65-72.
Vale, W.G., 2007. Effects of environment on buffalo reproduction. Italian Journal of Animal Science 6, 130-142.
Vale, W.G., Ribeiro, H.F.L., 2005. Reproductive patterns in buffaloes: puberty, estrous cycle, uterine involution and postpartum ovarian activity. Revista Brasileira de Reprodução Animal 29, 63-73.
Vieira, L.M., Rodrigues, C.A., Castor Neto, A., Guerreiro, B.M., Silveira, C.R.A., Moreira, R.J.C., Sá Filho, M.F., Bó, G.A., Mapletoft, R.J., Baruselli, P.S., 2014. Superstimulation prior to the ovum pick-up to improve in vitro embryo production in lactating and non-lactating Holstein cows. Theriogenology 82, 318-324.
Wadhwa, N., Kunj, N., Tiwari, S., Saraiya, M., Majumdar, S.S., 2009. Optimization of embryo culture conditions for increasing efficiency of cloning in buffalo (*Bubalus bubalis*) and generation of transgenic embryos via cloning. Cloning Stem Cells 11, 387-395.
Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10, 57-63.
Wu, Y., Lin, J., Han, B., Wang, L., Chen, Y., Liu, M., Huang, J., 2018. Proteomic profiling of follicle fluids after superstimulation in one-month-old lambs. Reproduction in Domestic Animals 53,186-194.
Yousaf, M.R., Chohan, K.R., 2003. Nuclear morphology, diameter and meiotic competence of buffalo oocytes relative to follicle size. Reproduction Fertility and Development 15, 223-229.
Zoheir, K.M.A., Abdoon, A.S., Mahrous, K.F., Amer, M.A., Zaher, M.M., Li-Guo, Y., El- Nahass, E.M., 2007. Effects of season on the quality and in vitro maturation rate of Egyptian buffalo (*Bubalus bubalis*) oocytes. Journal of Cell and Animal Biology 1, 29-33.