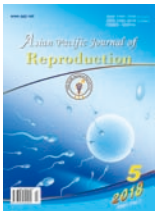

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Application of advanced reproductive biotechnologies for buffalo improvement with focusing on Egyptian buffaloes

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ABSTRACT

Many countries in the world consider the buffalo as a priority animal for the future, since it plays a pivotal role in human food sustainability. Even though Food and Agriculture Organization has termed the buffalo as an important undervalued asset, this species has yet to drive the same attention as cattle. Egypt has a wealth of buffaloes dispersed in small herds all over the country, so the efforts that have been made to improve their genetic background show little return. Contrarily, other countries concerned with buffalo improvement have already used a data recording system in buffalo herds, allowing to achieve a much faster improvement progress. This review intends to survey the existing information on the application of assisted reproduction techniques to improve buffalo productivity. The strength points that may help to improve buffalo production are identified, and the obstacles hindering the genetic improvement of Egyptian buffalo are characterized. Therefore, this work will gather information related to buffalo and compile it for an audience of researchers and specialists to enforce international collaboration for the development of buffalo production. Also, it will open the way for people interested in developing a future vision for buffalo potential, which will be helpful to close or minimize the biological gaps of buffaloes' researches.

1. Introduction

In recent years, the importance of buffalo species (*Bubalus bubalis*) has increased in tropical and subtropical countries because of its ecological and economic benefits, namely for its adaptability to thrive in the stressful harsh environments, converting poor-quality roughage into meat and milk, and also due to its working capacity[1]. As compared with the cattle, the metabolic energy used for milk production is presented with higher efficiency in buffalo than in cattle[2]. In addition, buffalo milk contains higher total solids (protein, fat and minerals) compared to cow milk (18%-23% *vs.*13%-16%, respectively). Thus, the buffalo is now foreseen as priority animal regarding the world's potential food supply, where it plays an axial role in human food sustainability, though buffalo importance has yet to receive the same attention and care as earned by cattle[3]. Hence, Food and Agriculture Organization has pointed the buffalo as an important yet undervalued asset[4]. Some Asian countries, such as India and Pakistan, are interested in improving the buffalo genetic potentiality, increasing its productivity, and reflecting the transformation of buffaloes into a dairy purpose animal; consequently, these countries fostered milk recording and a careful animal selection that contributed to setting up a core of animals with superior genetics. Therefore, in those countries, the dairy purpose buffalo become a pillar of economic development, while buffalo numbers keep increasing to link with the market economy. Even though Egypt is the only African country possessing a tremendous wealth of buffaloes, and the most important in the Middle East, the progress in the improvement of the species system is far from desirable. In spite that the buffalo represents 44.5% of

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the total population of large ruminants (8 870 022 heads) in Egypt; few measures were introduced to improve the production system and little efforts were made to improve their genetic potential. So, there is a large heterogeneity within buffalo populations in regard to milk and meat production even whether within the herd or under the same environmental conditions and management. In Italy, to the fulfillment of buffalo and its milk, recording systems were applied for the selection of dairy buffalo purpose, which can produce around 5 000 kg milk/270 days of lactation[5], namely the use of artificial insemination (AI), the high reproductive biotechnologyies used by farmers, high level of management, high genetic value of the herd and the progeny testing. Fortunately, buffaloes, unlike cattle, have not undergone massive selection and crossbreeding until only very recently. Therefore, adoption of genetic improvement programs should be of interest to increase the management of buffalo genetic resources.

2. Application of reproductive biotechnologies in buffaloes

There are many factors negatively affecting the reproductive efficiency of buffalo species and causing heavy economic losses to farmers. Major factors include delayed puberty/maturity, and consequently higher age at the first calving, long postpartum anestrus that elongates the calving interval, the lack of overt sign of heat (silent heat/sub-estrus), variable time of ovulation, breeding seasonality and low conception rate[6,7]. These factors received different prioritization of attention in different countries, or even between farms in the same country, and thereby it depends on the geographical location. Nevertheless, the reproductive efficiency of buffalo can be improved directly by implementing efficient management systems that may include various reproductive biotechnologies, which are a combination of assisted reproduction and other techniques. The fast pace of development in emerging reproductive biotechnologies has been used to improve the number of superior genotypes. AI and multiple ovulation and embryo transfer (MOET) can also be effectively applied to improve reproduction efficiency and enhance the production of genetically superior animals. These can help to reduce the generation interval and thereby accelerate the desired genetic improvement in buffaloes.

2.1. Buffalo semen cryopreservation

Cryopreservation of buffalo semen has gained its prominence as looking forward to upgrading production and reproductive efficiency, and allowing the widespread dissemination of valuable genetic material of superior sires by means of AI. The fertility rates following AI with cryopreserved buffalo semen are lower than in cattle, which is attributed to the low quality of cryopreserved buffalo semen[8]. Moreover, proposed protocols for improving semen production are different and not unified. Improvement of the technique for buffalo semen cryopreservation is therefore foreseen as a tool to enhance the breeding programs in this species, supporting the development of many studies on the major conditions affecting the semen freezability in buffalo. Different extending media have been tested for deep freezing of buffalo semen. Ziada *et al*[9] compared different extenders for frozen buffalo semen [Tris-citric acid fructose, laiciphoy (Lactose-citrate-phosphate), Triladyl (German commercial Tris-based extender), reconstituted skim milk powder and Laiciphos-478 (commercially produced by IMV, France)] and found that the reduction in motility due to the fact that freezing and thawing was lower in Tris (23.4%) and skim milk (36.4%) than in other extenders, and concluded that those diluents appeared to afford better protection against freezing hazards than all others. Another study reported that the whole buffalo milk achieved the highest post-thawing motility (53.65%) and viability (101.30) compared with Tris (46.94% and 78.65, respectively), egg yolk sodium citrate (46.84% and 83.13, respectively) and Begly diluents (45.90% and 100.60, respectively)[9,10]. Similarly, the whole buffalo milk-based diluent achieved higher post-thawing motility and viability index than sheep or goat milk[10].

Semen of buffalo bulls is usually collected in open places. So, to minimize bacterial contamination that might have detrimental effects on semen quality, lower the conception rate of AI and disseminate diseases among the healthy population, different antimicrobial agents have been tested in extended buffalo semen. Antibiotics combinations are more efficient for cryopreservation of cattle and buffalo bull semen. Traditionally, streptomycin and penicillin is the antibiotic combination that has been added to the diluents for buffalo bull semen[11]. Andrabi *et al*[12] referred to a combination of gentamycin, tylosin and linco-spectin (GTLS) which may be incorporated into a freezing extender of buffalo semen without compromising the post-thaw semen quality and *in vivo* fertility and that obtain a total aerobic bacterial count considerably lower in semen samples treated with GTLS compared with those treated with streptomycin and penicillin. This contrasts with the observed in cattle, where Gloria *et al*[13] concluded that GTLS failed to control bacterial growth in cryopreserved bull semen. Furthermore, GTLS in skimmed milk extender, compared to streptomycin/penicillin, did not improve the fertility of chilled buffalo bull semen[14]. However, the Certified Semen Services, considering the antibiotic components of semen extenders, have made it necessary to look for acceptable alternatives that must provide effective microbiological control. Ciprofloxacin has been found to be efficient for controlling bacteria contamination in buffalo semen extender without compromising the post-thaw semen quality and fertility[15]. Also, ceftiofur/tylosin and ofloxacin antibiotics can be safely added to bull semen extenders and both can protect insemination doses from bacteria that are resistant to other antibiotic combinations[13].

Egg yolk (EY) has been used traditionally as non-permeable cryoprotectant in buffalo semen[14], due to its content in low density lipoproteins that possess cryoprotective features[16]. Akhter *et al*[17] showed that quail EY at 5% and turkey EY at 10% in Tris citric acid extender offered advantages over 20% chicken EY in terms of *in vitro* post-thaw semen quality and *in vivo* fertility of buffalo semen. However, some studies also revealed that fully avian EY contains other substances, like high density lipoproteins, which

have a detrimental effect to sperm function[18]. It was suggested that these disadvantages in the use of EY can be minimized by replacing with egg yolk plasma (EYP) containing purified low density lipoproteins[18]. Recently, the effect of different concentrations of EYP in Tris citric acid extender on buffalo sperm quality during cryopreservation revealed that post thaw progressive sperm motility, viable sperm with intact acrosome, DNA integrity, and pregnancy rate were higher in 15% and 20% EYP compared to whole chicken egg yolk and other concentration of EYP[19]. The plasma membrane of the sperm cell is a key component in sperm fertilizing ability, and therefore must be maintained intact to keep sperm viable[20]. The spermatozoa plasma membrane contains high concentrations of polyunsaturated fatty acids, which are susceptible to reactive oxygen species (ROS), which irreversibly compromise sperm motility, the integrity of plasma membrane and DNA integrity, with a subsequent loss of sperm quality during freezing-thawing process[21]. Bovine semen has a natural defense system against the oxidative stress, albeit it is considered insufficient to prevent lipid peroxidation under cryopreservation process[22]. Compared to bovine, the buffalo sperm is more susceptible to cold and heat stresses which accompanied freezing and thawing process[23] and more sensitive to oxidative stress[24]. The addition of antioxidants to the freezing diluent may account to a protective effect against lipid peroxidation, thereby preserving the metabolic activity and cellular viability[25]. There are different antioxidant additives tested in standard buffalo tris extender, which neutralize the accumulation of free radicals triggering oxidation as it occurs during sperm cryopreservation, hence reducing the risk of damage to spermatozoa[26].

Trehalose is a non-reducing disaccharide maintaining the osmotic pressure of the diluents[20], causing cellular osmotic dehydration before freezing, and thus decreasing the extent of cell injury induced by intracellular ice crystallization[27]. The beneficial effect of the trehalose on the post-thawing viability of buffalo semen has been reported by different authors[28]. The addition of 100 mM trehalose to buffalo semen extender improved the post thaw sperm motility, viability and acrosomal integrity while reducing sperm DNA damage, which improved the *in vitro* fertilization rate[29]. However, a high trehalose concentration in Tris-based egg extender has a detrimental effect on post-thawing motility and plasma membrane integrity of buffalo semen[30].

Several amino acids have been detected in seminal plasma and play an important role in preventing oxidative damage to spermatozoa, by coping with ROS levels by the increase of intracellular activity of antioxidants[31]; thus, amino acids might be successfully used as a non-permeating cryoprotectants, exploring its positive effects on post-thaw sperm motility and protection of the sperm membrane integrity, with subsequently increasing the fertilizing capacity[32].

Cysteine is a precursor of glutathione (GSH) and has been shown to penetrate the cell membrane easily, enhancing the intracellular GSH biosynthesis and protecting the membrane lipids and proteins[33]. Cysteine has an additional cryoprotective effect on the functional integrity of axosome and mitochondria, improving post-thawed sperm motility[34]. Incorporation of cysteine with ascorbic acid in standard tris-fructose-egg yolk-glycerol extender improves sperm quality parameters, reduces enzyme leakage, and ultimately advances cryopreserve ability of buffalo semen[22].

Hypotaurine is a precursor of taurine, which exists in the mammalian spermatozoon and it is essential for several sperm functions, such as motility, capacitation, fertilizing ability and early embryonic development[35]. Taurine has positive effects on sperm membrane function and the structural integrity of the acrosome membrane[36]. The addition of a mixture of hypotaurine, trehalose and cysteine to Tris extender of buffalo semen significantly improved post-thawing sperm motility, viability index and maintained acrosomal integrity, besides exerting valuable effects in *in vitro* fertilizing potential[36]. This suggests the existence of a synergistic action among trehalose, cysteine and hypotaurine.

L-carnitine, a vitamin-like amino acid with powerful antioxidant ability[37], acts as cofactor accelerating the transport of fatty acids into the mitochondria to generate adenosine triphosphate, and is an important fuel source for sperm motility[38]. Moreover, it protects sperm DNA and prevents protein oxidation and damage[39]. The presence of *L*-carnitine at a concentration of 0.05 mg/mL in the extension medium enhanced the frozen spermatozoa quality in buffaloes by preserving the plasma membrane and mitochondrial functional integrity and potentiating its fertilizing capacity[40].

Glutamine, an amino acid having an extracellular mechanism of action[31], may be added to buffalo semen extenders to improve the quality of post thaw spermatozoa with the subsequent increase of fertility rate[41]. However, only glutamine levels between 20 mM to 60 mM showed beneficial effects, while lower or higher doses showed toxicity for spermatozoa and caused a significant reduction in sperm motility and viability[42].

Melatonin is an indole derivate playing multiple actions in the regulation of the reproductive functions[43]. It has also been demonstrated that melatonin metabolites have the ability of sweeping ROS[44,45], besides, the fantastic enhancing role on sperm capacitation increases its fertilizing capacity[46]. The beneficial functions of the melatonin on post thawing buffalo semen characteristic are dose-dependent. The addition of 0.10 mM and 0.25 mM melatonin to buffalo semen diluents is sufficiently effective to enhance its fertilizing ability *in vitro*[47] and increases the conception rate[48]. These effects were associated to melatonin role in the preservation of the mitochondrial dense structure, arrangement of the spermatozoa, improvement semen quality and reduction of cryo-damage to the spermatozoa[49].

Selenium is an essential trace nutrient and an integral part of glutathione peroxidase enzyme which protects cell internal structures against ROS[50]. Selenium deficiency has been linked to reproductive problems with an impaired semen quality in different animal species, and its supplementation has been reported to improve reproductive performance[51]. Addition of 1.0 to 2.5 μg/mL of selenium to buffalo semen extender significantly improved post thaw semen motility, sperm viability and membrane integrity, lowered DNA damaged sperms[52] and significantly increased the conception rate[53]. However, selenium supplementation to semen extender at concentrations $\geqslant$4 μg/mL had deleterious effects on sperm parameters[52].

2.2. Estrus synchronization as a tool for improvement of fertility

AI is often implemented in combination with selection programs as a most important technique widely used for dissemination of superior genetic material of males to improve the efficiency rate of genetic selection. AI programs used in buffaloes have a limited expression, representing less than 10.0% of breeding (only 5.0% in Italy, 3.7% in Azerbaijan, 0.3% in Egypt, and 0.1% in Romania). However, in Bulgaria where it can be found in the largest cooperative state farms, AI is applied to 80% of the buffaloes[54]. Estrus detection is a prerequisite for AI, as it would help to predict the appropriate time of ovulation for timed insemination and increase conception rate[55]. However, estrus behavior in buffalo has a weaker expression than that in cattle, especially in heifers, thus representing one of the challenges in the application of AI in buffaloes. The incidence of silent heat or so called sub-estrus reaches 70% in buffaloes and estrus is often undetected under natural field conditions[56], which adversely concurs to huge economic loss. The acceptance of the buffalo cows to bulls is considered the most reliable indicator for estrus detection[57]. Still, it is possible to monitor follicular development for estrus and ovulation using the ultrasonography[58]. Recently, electronic radio telemetry has also been used with 100% accuracy for estrus detection in synchronized buffalo heifers[59]. However, estrus synchronization has been developed to intensify the estrus and overcome the estrous detection with improving conception rate[60].

Buffaloes are characterized by seasonal reproductive activity; they show distinct seasonal variations in displaying estrus, even though the proportion of animals displaying estrus during the shorter day period is greater than in the longer day period. Buffaloes seasonality affects the efficiency of the synchronization protocols[61]. Estrus synchronization protocols in buffalo are based on those existing for cattle, either by inducing premature luteolysis with prostaglandins or by prolonging the luteal phase using progestogens. Although the administration of single dose prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is effective and economical for estrus synchronization in buffaloes[62,63], it has a limitation because it works only in the presence of an active corpus luteum (CL). The application of gonadotropin-releasing hormone (GnRH) or analogs along $PGF_2\alpha$ strengthens the response because it causes ovulation or luteinization of large follicles present in the ovary, and subsequently synchronizes the recruitment of a new follicular wave[64]. This protocol enhances estrus detection and enables to precisely control ovulation time (synchronization of ovulations), therefore, facilitating the use of fixed timed of AI (FTAI). The use of hormonal protocols associated with FTAI presents more advantages and is practical to overcome the problem of estrus detection in buffaloes, especially during the seasonal anestrus[65].

One of the most popular protocols used over the last decade for estrus synchronization and FTAI in cattle and buffaloes is Ovsynch—initially developed to involve sequential injections of GnRH on Day 0, $PGF_2\alpha$ analogue on Day 7, GnRH on Day 9, and timed of AI on Day 10[64]—that results in fertility rates similar to those of AI at estrus detection[66], while eliminating the practical problems of heat detection in buffalo[67]. Even though it has shown satisfactory results in favorable season[68], it presents low conception rate when applied in summer, because Ovsynch protocols require that animals are cyclic[69]. Furthermore, there is 20%-40% failure of ovulation recorded in buffaloes following Ovsynch protocol[69], which may result from the absence of a dominant follicle at the moment of the first GnRH injection and subsequently to CL absence at the day of $PGF_2\alpha$ injection[70]. Lower circulating progesterone, which subsequently delays ova maturation at the time of insemination, may be another cause for low conception[71]. Therefore, many researchers suggested that administration of exogenous progesterone in the form of controlled intravaginal releasing device (CIDR) along Ovsynch-FTAI protocols would prevent the onset of premature estrus allowing formation of CL with normal life span following CIDR removal[71]. So, the Ovsynch+CIDR protocol presents better estrus response and higher pregnancy rate in buffaloes during the breeding season[72] and in postpartum anestrous buffalo[73]. To enhance the reproductive efficiency in the postpartum buffalo during the summer season, a new synchronization method called Doublesynch can also be used. This protocol includes the administration of an additional $PGF_2\alpha$ injection 48 h before the beginning of Ovsynch protocol[74]. The highest pregnancy rate (58.0%) was achieved in Doublesynch compared to control group (39.0%), suggesting that Doublesynch protocol can be successfully used in buffaloes during summer[75]. Furthermore, authors found the service period in Doublesynch group was 12 days shorter than control that improved the economics of dairy farm. Similarly, Mirmahmoudi and Prakash[76] concluded the Doublesynch protocol produced efficient synchronization of ovulation twice, *i.e.*, after the first and second GnRH administrations and the anestrus buffaloes had pregnancy rates as high as those recorded in cycling buffaloes after treatment with the Doublesynch protocol (55.0% and 60.0%, respectively). The authors elaborated that this finding may be attributed to the high release of luteinizing hormone (LH) following the first GnRH injection due to a low progesterone environment brought about by the additional $PGF_2\alpha$ injection administrated 2 days prior to the first GnRH, leading to most of the animals ovulating after the first GnRH injection, and hence, creating the optimum follicular size for ovulation and conception to occur at the second GnRH injection.

The efficacy of melatonin implants followed by CIDR treatment was tested for mitigating the adverse effect of summer stress on the ovarian activity in anestrus lactating buffaloes. Ramadan *et al*[77] concluded that CIDR treatment preceded by melatonin improved the reproductive performance in lactating buffaloes, which achieved a higher conception rate than control.

Many attempts to modify Ovsynch protocols were performed, in which estradiol benzoate (EB) was injected after CIDR removal in nulliparous and multiparous buffalo[78]. It has been concluded that the administration of EB in conjunction with CIDR might be better and preferred to that of GnRH, as it improves estrous intensity and generates greater ovulation rates, which could be implemented with FTAI program with best pregnancy rates. EB-based protocols have several advantages compared with GnRH-based protocol in

estrus synchronization in buffaloes, which include its lower cost, an utmost control of follicular growth plus the occurrence of estrus, a better uterine tone, and subsequently a relative ease insemination, and likely creates a better uterine environment for embryonic development[79]. Furthermore, there is evidence that EB obtains an increased LH release and greater induction of ovulation[80]. However, recently, neither Yousuf *et al*[78] nor de Carvalho *et al*[81] were able to find significant differences in the induced follicular response and ovulation when comparing EB and GnRH in progesterone based synchronization protocol in buffaloes.

When implementing FTAI protocols in the buffalo, it is necessary to know the time of ovulation to optimize AI efficiency. Ovulation ranges from 65-75 h after CIDR removal in buffalo[82]. To enhance the fertility rates, the optimal time of AI is 48-60 h[83], and the double inseminations execute a significantly higher pregnancy rate than single insemination in buffalo treated with CIDR-GnRH protocol[84].

2.3. Use of sexed buffalo semen for AI

Sexed semen in bovines increases the effectiveness of AI, deviating the sex ratio in favor of females and the rapid expansion of dairy herds carrying productive traits of high genetic values. Buffalo sperm can be sorted into X and Y chromosome-bearing spermatozoa on the basis of physical differences and DNA contents through cell flow cytometry sorting separation, with similar success as in cattle[85]. Sexed sperm in buffalo used for AI with X-sorted have 90% purity and 82.8% accuracy when applied in the field[84]. The application of sexed sperm in buffalo breeding plans in herds would be economically feasible, and would target the upbringing of heifers with distinctive potential genotype as replacement for low productivity buffalo cows. Regarding to the rates of pregnancy, the practical use of AI with sexed semen in buffalo heifers gave satisfactory and promising results comparable with conventional non-sexed semen[86]. Lu *et al*[84] showed that higher pregnancy rates could be obtained with sexed buffalo semen using AI than unsexed semen (69.7% *vs.* 66.5%). However, the results of AI with sexed buffalo semen may be dependent on many factors, and more studies on this topic are foreseen.

Mediterranean buffaloes showed a pregnancy rate of between 30% and 50% when inseminated with sexed semen[87]. It has been demonstrated that a breed influence on the success of sex sorted semen existed: the pregnancy rate obtained was higher for Murrah bulls (52.5%) than for Nili-Ravi's (46.1%) or water buffalo bulls (48.5%), suggesting that the use of Murrah bulls might be preferable for sexed semen production[87]. This was supported by Lu *et al*[84], who found lower conception rates with sexed sperm derived from Nili-Ravi bull (55%) compared with Murrah's (83.6%), which was attributed to differences in the morphology and genetic background of sperm and their ability to withstand the sexing procedures. Alike the report in cattle[88], individual differences in the pregnancy rates amongst donors of sexed semen were recorded in buffaloes[87]. The authors attributed this variation to differences in morphology and genetics of sperm and their ability to withstand the sexing procedures[87]. Thereby, to amplify the output of AI using sexed semen technology, breeding bulls should be beforehand selected carefully.

Parity is an important factor affecting fertility when sexed semen is used in Holstein cows. The pregnancy rate in parous cows was lower than in heifers (60% *vs.* 80%, respectively)[89]. Thus, for economic considerations, the sexed semen was more often used for AI in heifers[90]. However, no difference in pregnancy rate was seen in nulliparous and parous buffalo cows[87], suggesting that parity may not exert the same effect on the fertility as it does on dairy cows. Furthermore, the conception rate in heifers was lower than in parous buffaloes when synchronization and FTAI were used, but it was similar when they were inseminated at natural estrus[91].

The site for sperm deposition plays an important role in the fertility of sex-sorted semen[86]. Campanile *et al*[86] recorded a significantly higher pregnancy rate when sexed buffalo semen was deposited into the body of the uterus rather than in the uterine horn (45.5% *vs.* 32.3%). Others' reports demonstrated that the success of AI with sexed sperm was enhanced by using a special catheter—Ghent insemination device[92], enabling the gentle deposition of spermatozoa close to the utero-tubal junction in cattle[93] and buffalo[85,86]. The optimum number of sexed sperm in the inseminating dose, allowing to obtain promising pregnancy rates (49.8%), was defined as 4 million for the Italian buffalo[94]. Nevertheless, others registered similar pregnancy rates in buffalo heifers using 2 million live sorted sperm per dose and non-sexed semen (38.8% *vs.* 37.7%)[86].

2.4. Embryo transfer–a tool for genetic improvement

MOET is a technique by which embryos are collected from a superovulated donor female and transferred to recipient females that serve as surrogate mothers for the remainder of pregnancy. MOET technique applied in buffalo is essentially a replicate of those used in cows, and involves multiple ovulation in females by administration of gonadotropins in the luteal phase of the estrous cycle, followed by induction of estrus, *in vivo* fertilization, non-surgical recovery of embryos and transfer into suitable synchronized recipients. This technique applied for embryo production exploits the genetic potential in females to accelerate the multiplication of superior animals. It consists of several steps, namely the selection of donor and recipient females, superovulation and the concurrent synchronization of estrus between donor and recipients, the recovery of embryos and afterwards its examination and classification, and finally the transfer of suitable embryos. Embryo transfer enhances the upgrade of herd genetics by careful pairing of donors and bulls. Also, this technology reduces the generation interval and opens the possibilities for enlarging progeny population of high genetic merit dams in the nucleus breeding program[95], of particular interest for buffaloes, allowing to increase the selection intensity over desired production traits. Ultimately, MOET is used to produce genetically selected AI sires from proven cows and bulls[96].

For a few years, new genomic techniques have been used increasingly to select embryo donors, especially for selection of

dairy dams for super-stimulation, where a genomic analysis is becoming essential[97]. Many breeders have embraced genomic selection and routinely used genomic estimated breeding values when purchasing semen or deciding which cows and heifers merit investment in reproductive technologies such as MOET. At the same time, AI companies are aggressively using genomic testing to determine which young bull to purchase, marketing semen to dairy producers, and identifying elite females that can make positive genetic contributions to the next generation. By increasing the accuracy and the intensity of selection and shortening the generation interval, the rate of genetic progress for economically important dairy cattle traits can be almost doubled. Moreover, when a breeder identifies genetically superior females using genomic testing, these animals usually become part of a MOET based program, after reaching sexual maturity[98].

The main goal of the dairyman is not to produce a sire for potential use in AI, but to produce females of high quality to be used for the next generation in dairy herds. Therefore, dairymen utilize sires that meet their selection criteria and every female in the herd is bred to create a potential replacement. Now, by the aids of MOET, every dairyman can select females or even groups of females of high production to use as replacement generators in their herds. On the flip side, females of lower production can be utilized for other aims rather than create a female replacement, that is, they can be used as recipients for embryos.

However, in buffalo, the production of a sufficient number of viable embryos with a high probability for producing multiple transfer pregnancies has been limited, in spite that the implementation of MOET in this species started three decades ago[99]. The results achieved so far have been modest and rather distinct from cattle because of some species peculiarities. The efficiency of embryo production in buffalo has not improved much over the years and this is due to many factors.

Buffalo ovaries are smaller than cattle's, and the corpora luteas are deeply embedded in the ovarian stroma, so the ovarian structures are difficult to palpate per rectum[100]. The smaller size of the corpora lutea is one of the reasons for lower progesterone production in bovine species[101]. Also, the primordial follicles pool on the ovary is smaller in buffalo than in cattle[102], so the antral follicles found at all stages of the estrus cycle are fewer in buffalo than in cattle[103]. Furthermore, a high frequency of deep atresia of follicles is reported in the buffaloes' ovary than in cattle's[104]. The reproductive efficiency of buffaloes shows wide variation throughout the year in relation with climate and photoperiod[105], particularly depending on melatonin secretion which plays a pivotal role[106]. Compared with cattle, the homosexual estrus activity in buffaloes is not as pronounced as in cows[107]. Moreover, the estrus is often silent, and the circulating levels of estrogen have been reported to be low when compared with cattle[108], and this reflects on silencing estrus expression[109]. Plasma inhibin levels in relation to steroids and gonadotrophins during estrous cycle range between 391.25 and 631.97 pg/mL during various phases of the estrous cycle and are found to be higher than that reported in cows[110]. Inhibin suppresses the production and/or secretion of follicle-stimulating hormone (FSH) through negative feedback at pituitary level[111]. Plasma estradiol, progesterone and FSH concentrations and FSH/LH ratio are affected by weather in buffalo[112]. The concentration of these hormones is lower in summer season compared to cooler months[113]. These factors could account for limiting the response of MOET technology in this species, in terms of low ovarian super-stimulation response and transferable embryo recovery.

2.5. Ovarian super-stimulation in buffalo

For superovulation of donor buffalo cows, the treatment protocols usually rely on the administration of FSH and equine chorionic gonadotropin (eCG). The hormonal treatment starts in the mid-luteal phase (8 to 12 days) of the donor's estrous cycle. The eCG is a complex glycoprotein with a prolonged half-life (> 40 h) in the circulation and only a single injection of 2 000-3 000 IU is required to elicit super stimulatory response[114,115]. Conversely, the biological half-life of FSH has been reported to be ≤5 h[116]; thereby multiple injections are necessary to elicit optimal ovarian stimulation, given twice daily, over 4-5 days, with a total dose of 40-50 mg[117]. It has been shown that twice daily injections of FSH induced a greater super stimulatory response than once daily treatments[118]. However, in field trials, 40 mg FSH divided into eight equal doses stimulated a lower ovarian response than 40 mg FSH administered in eight decreasing doses[100]. Similarly, the total embryos recovered and the numbers of transferable embryos were greater in the gradually descending dose of FSH than in the constant or a steeply descending dose[119]. In FSH-treated buffalo, the supplementation of GnRH at 8-12 h after standing heat produces more transferable embryos was compared with controls or buffaloes treated at standing heat[120]. Another study refers to a significant higher proportion of transferable embryos recovered in buffaloes treated with LH as compared to GnRH[121]. It was also demonstrated that the application of estradiol-17 β and human chorionic gonadotropin concurs to improve the ovarian response, and consequently ovulation and fertilization rates in cycling and anestrous buffaloes of different breeds[122,123].

Comparatively, FSH has been found to be preferable as super-stimulatory agent than eCG, as producing more CL and recovered embryos of high quality[115]. Some problems resulted from eCG administration, including the presence of anovulatory follicles that secrete estradiol far in excess of the normal preovulatory concentration which is in combination with a low concentration of progesterone secreted during the early luteal phase of the super-stimulator cycle, leads to an undesirable uterine environment for embryos. Furthermore, after ovulation, eCG is still present in the circulation and might have a deleterious effect on the quality of embryos by stimulating steroid secretion. On the other side, in addition to its expensive cost, compared to eCG, the FSH treatment presents as disadvantages of the excessive handling associated with frequent injections, which may be stressful to donor animals and may result in a reduced super-stimulatory response[115,124]. However, researchers successfully simplified protocols of super-stimulation to reduce donor handling and improve response, particularly in fierce animals. The combination of FSH with a slow release carrier that

could induce a super-stimulatory response over several days has been tested. A single injection of 5 mg FSH diluted in 3.2% gelatin protein as a vehicle, once per day, allowed to cut animal handling in half[118]. Moreover, the use of the same protocol with 50 mg FSH as one injection gave results similar to that of 5 days, twice-daily treatment protocol[125]. Aluminum hydroxide gel, polyvinylpyrrolidone and hyaluronan are other beneficial carrier, all of which would release its carried molecule slowly over a period of several days; so, when used in combination with FSH and administered as a single injection, it allowed to induce ovarian super-stimulation[125,126].

Super-stimulation remains elusive and the less predictable step in the process of embryo production in buffalo[127]. The vast differences in the ovarian response to gonadotropin stimulation are still a major problem in MOET programs. In most cases, failure to respond to the first super-stimulation attempt will likely result in failure in subsequently treatment regimens. Moreover, ovarian responses and embryo recovery vary widely with age, parity, breed, season of the year, nutrition, milk yield status, lactation number, type of the super-stimulatory agent and the stage of the estrous cycle at which the treatment is initiated[128].

It has been found that the ovarian antral follicular population in cattle was associated with the circulating concentrations of anti-Müllerian hormone (AMH), insulin and insulin-like growth factor[129]. AMH, produced by granulosa cells of antral follicles, is used as a reliable endocrine marker of ovarian reserve[130]. A strong positive relationship between ovarian hyper-stimulation and embryo production was reported in dairy cattle[131,132] and buffalo[133]. Thus, the concentration of circulating AMH might help to forecast the response of ovarian super-stimulation. Moreover, AMH could be utilized as a tool instead to ultrasound for ovarian follicular count during the estrous cycle[132,134]. Early studies indicated by ultrasonography scanning have proven the presence of a pattern of one to four follicular waves during the buffalo estrous cycle and the existence of seasonal variations in follicular dynamics[103,135]. The ovarian response, total recovered embryos, the number of transferable embryos and the pregnancies achieved after eembryo transfer, all have been found to differ during monsoon periods, being significantly higher in winter than summer[136]. Moreover, the ovarian activity is greater in the cooler months with short day lengths than in summer[137]. The percentages of excellent and good quality oocyte were significantly higher during winter and spring than summer and autumn[137,138]. During the dry hot season, buffaloes show higher prolactin secretion and it is thought to be one of the reasons for the poor reproductive performance during these months[139]. Compared with the wet cool season, the dry hot season was marked with a progressive decrease in the number of small follicles and a rapid disappearance of follicles from the medium size during the super-stimulation treatment. Also, it was observed that during hot season, the small and medium size ovarian follicles, which were present at the time of super-stimulatory treatment, were faster turnover to large follicles and it was found to be adversely affected follicular maturation and luteal function, besides detrimental effects to the embryo quality[103].

The ovarian response, measured by the number of CL, total number of recovered embryos and transferable embryos, was significantly higher in buffaloes when the super-stimulatory treatments were initiated in the absence of dominant follicles than in animals having dominant follicles[103,121,140]. It has been suggested that the presence of a dominant follicle which developed during follicular wave at the time of initiation of gonadotrophin treatment suppress prevents the inferior neighbouring follicles to the emergence of the next follicular wave thereby increasing the atresia of recruitable follicles. Thus, the presence of a dominant follicle at initiation of gonadotrophin treatment exerts a negative effect on subordinate follicles and decreases the super-stimulatory response[103,141]. Circulating progesterone levels on the day of initial FSH injections are positively correlated with the ovulation rate in buffalo[140] and the high concentration indicates ovary has functional CL.

Embryos produced from super-stimulated cows are more advanced in development relative to those from untreated, natural cycles[142]. Thus, higher pregnancy rates in cattle are observed when recipients and donors coincide in estrus or the former anticipated 12 h compared to donors[128], whereas pregnancy rates decrease when recipients show estrus 12-24 h before the donors[142,143]. Conversely, in buffalo, high pregnancy rates are achieved when donors and recipients are in estrus within 12 h of each other; when synchrony exceeded 12 h, no embryos were successfully implanted[144,145].

Pregnancy rates are similar whether the embryos are transferred into the right or the left uterine horn, with no significant effect of the site of the transfer, whereas the rate of conception increased as the CL size of recipient increased[145], suggesting that the suitability of recipients depends on the size of a functional CL, which tended to influence the pregnancy rate, whereas, there is no difference in the incidence of right versus left side ovulations. Likewise, the type of estrus (spontaneous or induced) or the stage of embryo development (early morulae or compact morulae) had no effect on pregnancy, whereas embryo quality can be done, *i.e.* transfer of embryos with excellent and good quality resulting in high pregnancy rate than embryos of inferior quality[145].

2.6. Cryopreservation of buffalo embryos

Embryos produced either by MOET or *in vitro* fertilization can be preserved and stored for many years at low temperature (-196 ℃) using liquid nitrogen. These embryos can be thawed at any time in the future to be transferred to recipient females under desired optimal conditions. Embryo cryopreservation also provides global genetic transport for maternal germplasm allowing increasing the herd genetic selection pressure. Moreover, the breeding line regeneration or proliferation for genetic resource rescue can be achieved[146]. Many authors have tried to simplify the procedure of embryo preservation in buffaloes and investigated factors affecting the success of embryo freezing. To study the effects of cryoprotectants on the embryo viability, different cryoprotectants (glycerol, ethylene glycol or dimethylsulfoxide) were added in a 3-step (0.50 M; 1.00 M; 1.50 M) or 6-step (0.25 M; 0.50 M; 0.75 M; 1.00 M; 1.25 M; 1.50 M) procedure using increasing concentrations of cryoprotectant in phosphate buffer saline at 10 min intervals. After freezing and

thawing, each cryoprotectant was withdrawn and removed gradually by 3 or 6 stepwise reverse steps of addition. Each removal protocol was performed for embryos in each cryoprotectant. The viability of the embryo was evaluated by its capacity to subsequently develop and by using fluorescence vital stain[147]. The 6-step addition of glycerol produced buffalo embryos with highly brighter fluorescence than 3-step and it was also demonstrated that the blastocyst stage is more viable than the morulae's. Similarly, the gradual removal of the cryoprotectant in the embryo freezing solution after thawing would avoid zona pellucida damages. Some experiments were developed using conventional equilibrium methods and freezing machines for cryopreservation of the buffalo embryos has been reported[147,148]. Glycerol was found to improve survival of bovine embryos after freezing and thawing[115], although it was with very low pregnancy rates: when frozen embryos in 1.4 M glycerol were transferred to recipients, the pregnancy rate was 28%, and only 82% of pregnancies are live births[148].

Vitrification is a new technique alternative to conventional methods for embryo freezing. It was defined as a physical process in which a highly-concentrated solution of cryoprotectants jellifies during cooling, without the formation of ice crystals[149]. Vitrification greatly simplifies the process of cooling, avoiding physical damage to embryos, and lessens the chilling injury of embryos as it passes through critical temperatures very rapidly[150]. However, the embryos cryopreserved by vitrification may still be injured because of the toxicity of cryoprotectants, extracellular ice fracture, and adverse osmotic effects[148]. The results achieved in experiments carried out so far give reason to believe that it is possible to create banks for deep frozen buffalo embryos. These banks could be used successfully for conservation of genetic resources in buffaloes, introduction of new breeds and rapid dissemination of high producing buffalo genotypes[151]. The application of vitrification to field conditions reduces the equipment needed and technical skill required and provides considerable cost and time-saving per embryo transferred[152].

3. Conceptualization to promote Egyptian buffalo productivity

The improvement of production performance of Egyptian buffalo has become an urgent need, but there are impediments holding the project. For relevant background, the first challenge to the improvement of buffalo production efficiency in Egypt is related to the system of buffalo distribution and breeding. The overwhelming majority (97.5%) of the buffalo population in Egypt is dispersed all over the country, with farmers in small herds (two to five heads) [54,153]. Such herds are suffering from malnutrition, poor housing, lack of proper veterinary services and impaired management practices. Furthermore, the supporting services of AI, milk recording, genetic evaluation and milk marketing systems are unavailable[154]. Moreover, most buffaloes at small holders are bred naturally with untested bulls, *i.e.* bulls not selected for production improvement and not examined for reproductive diseases. This favours the low fertility rates and the spreading of semen borne diseases, leading to high economic losses. Other side of the problem is represented by buffalo herds with average size of 9 heads per household and located in peri-urban areas. Compared to the smallholders, these premium herds are characterized by higher milk production, longer lactation length[153] and have been enjoying a relatively good level of nutrition and managerial situations, due to their high milk revenues. Unfortunately, these stellar buffalo cows are often discarded and slaughtered after the end of the lactation period. Consequently, there are a lot of losses in the best buffalo cows that could have been used for genetic breeding programmes. On the other side, many farms are specialized in intensive milk production and have more than 50 heads of dairy buffalo per farm, representing larger commercial herds with better management system[153]. The milk recording system under this production system is practiced mainly to improve the management routine of the farm. Some farms have milk processing plant and feed milling machine, subsequently, herds in this system of production show better levels of appropriate care and attention and better overall management.

In Egypt, there are two main institutions that produce bulls and frozen semen for insemination: Animal Production Research Institute and the General Authority for Veterinary Services. Animal Production Research Institute selects young buffalo bulls provisionally according to dam milk yield, but the final decision is based on the bull's physical fitness and its semen quality. The General Authority for Veterinary Services selects buffalo sires from the market according to their body conformation, physical fitness and semen quality[154]. In addition, no connection links between recording, genetic evaluation and AI institutions, consequently no genetic improvement programs are practiced in Egypt[154]. As productivity traits are not yet a primary concern for selection of those sires, the genetic improvement did not materialize concretely, and the problem is settled by importing Italian frozen buffalo semen for genetic improvement. The hazardous use of imported frozen semen, without awareness and precautions, might compromise the genetic performance of native buffalo breeds, in spite of an existing national demand to keep and conserve the genetic material of native breeds. The other side of the problem is evidenced by the lack of a strategy for communication and cooperation between the authorities involved. For example, the Cattle Information System/Egypt of the Cairo University has recently recorded many herds of different size (small: one to five animals; medium: six to twenty; and larger herds). Data analyses monthly produced herd summaries and individual milk yield information, along with the genetic evaluation performed. However, these outputs are regrettably inactivated, and no sire or cow directory has been published due to the lack of links between milk recording and AI organizations.

In the preceding paragraphs, we tried to embody and characterize the problems that hinder the improvement of buffalo productivity while in this section we visualize future proposal for the advancement and development of Egyptian buffalo. This proposal puts the first foundation stone for the improvement and increase of buffalo productivity by giving the priority to small holders, village farms throughout Egypt. The presence of sizeable numbers of highly

lactated buffalo cows and the presence of reasonable and suitable high quality buffalo bulls with high breeding value for propagating their valuable genes through AI are the main targets of the proposal. Conservation of genetic material (semen and embryos) is also one of the objectives, as well as the establishment of a small herd of high milk yield that may be used in the future as a nucleus herd for genetic improvement to supply and strengthen AI centers with high quality frozen semen and embryos.

4. Conclusion

The potential of buffalo has made it a favorite future animal for tropical and sub-tropical areas, so some attention should be paid to buffalo for improving its productivity. Many countries such as Italy, India and Pakistan are concerned with the buffalo through encouraging the farmers and breeders for milk recording and help them in the selection activity which was reflected in the improvement of genetic potentiality with productivity increases of their buffalo herds. With regard to developing countries that are suffering from a shortage of animal resources, in spite of possessing a wealth of buffalo herds, they should be working to improve their productivity by drawing on the experiences of other countries that predecessor in this area. The application of advanced reproductive biotechnology concurrently with genetic programs could be helpful to achieve this target within short time.

Conflict of interest statement

The author declares that there is no conflict of interest.

References

[1] Efrain C, Edmundo E, Diego S. Background and situational diagnosis of raising buffalo (*Bubalus bubalis*) in Valle Sacta, Cochabamba, Plurinational State of Bolivia. Proceedings of the 11th World Buffalo Congress; 21-26 November 2016; Cartagena, Colombia.

[2] Pradhan K, Bhatia SK, Sangwan DC. *Relative rumen ecosystem and nutrient digestibility in cattle and buffalo fed high fibrous diets*. Hisar, India: Haryana Agicultural University; 1991.

[3] Borghese A, editor. *Buffalo production and research*. Rome, Italy: FAO of the United Nations; 2005.

[4] FAO. *Water buffalo: An asset undervalued*. Bangkok, Thailand: FAO Regional Office for Asia Pacific; 2000, p. 1-6.

[5] Borghese A, Mazzi M. Buffalo population and strategies in the World. In: Borghese A, editor. *Buffalo production and research*. Rome, Italy: FAO of the United Nations; 2005, p. 1-40.

[6] Suthar VS, Dhami AJ. Estrus detection methods in buffalo. *Vet World* 2010; **3**(2): 94-96.

[7] Zicarelli L. Can we consider buffalo a non precocious and hypofertile species? *Ital J Anim Sci* 2007; **6**(2): 143-154.

[8] Andrabi SMH, Ansari MS, Ullah N, Anwar M, Mehmood A, Akhter S. Duck egg yolk in extender improves the freezability of buffalo bull spermatozoa. *Anim Reprod Sci* 2008; **104**(2-4): 427-433.

[9] Ziada MS, Darwish GM, Mohammed KM. Freezability of buffalo semen concerning different diluents, freezing programs and thawing regimens. *Alex J Vet Sci* 1998; **14**: 85.

[10] Mohammed KM, Ziada MS, Darwish GM. Practical trials for freezing semen on buffalo and friesian bulls: Effect of various regimens of freezing different milk extenders and types of straws packages on post-thawing semen characters. *Assiut Vet Med J* 1998; **39**: 70.

[11] Andrabi SMH, Ahmad N, Abbas A, Anzar M. Effect of two different antibiotic combinations on fertility of frozen buffalo and Sahiwal bull semen. *Pakistan Vet J* 2001; **21**(4): 166-169.

[12] Andrabi SMH, Khan LA, Shahab M. Isolation of bacteria in semen and evaluation of antibiotics in extender for cryopreservation of buffalo (*Bubalus bubalis*) bull spermatozoa. *Andrologia* 2016; **48**(10): 1166-1174.

[13] Gloria A, Contria A, Wegherb L, Vignolaa G, Dellamariac D, Carluccio A. The effects of antibiotic additions to extenders on fresh andfrozen–thawed bull semen. *Anim Reprod Sci* 2014; **150**(1-2):15-23.

[14] Akhter S, Sajjad M, Andrabi SM, Ullah N, Qayyum M. Effect of antibiotics in extender on fertility of liquid buffalo bull semen. *Pakistan Vet J* 2007; **27**(1): 13-16.

[15] Akhter S, Ansari MS, Rakha BA, Andrabi SM, Qadeer S, Iqbal R, Ullah N. Efficiency of ciprofloxacin for bacterial control post-thaw quality, and *in vivo* fertility of buffalo spermatozoa. *Theriogenology* 2013; **80**(4): 378-383.

[16] Su L, Li X, Quan X, Yang S, Li Y, He X, Tang X. A comparison of the protective action of added egg yolks from five avian species to the cryopreservation of bull sperm. *Anim Reprod Sci* 2008; **104**(2-4): 212-219.

[17] Akhter S, Rakha BA, Ansari MS, Husna AU, Iqbal S, Khalid M. Evaluation of quail and turkey egg yolk for cryopreservation of Nili-Ravi Buffalo bull semen. *Theriogenology* 2017; **87**: 259-265.

[18] Akhter S, Ansari MS, Rakha BA, Andrabi SMH, Khalid M, Ullah N. Effect of low density lipoproteins in extender on freezability and fertility of buffalo (*Bubalus bubalis*) bull semen. *Theriogenology* 2011; **76**(4): 759-764.

[19] Hussain Shah SA, Hassan Andrabi SM, Ahmed H, Qureshi IZ. Chicken egg yolk plasma in tris-citric acid extender improves the quality and fertility of cryopreserved water buffalo (*Bubalus bubalis*) spermatozoa. *Theriogenology* 2017; **89**: 32-40.

[20] Aboagla EM, Terada T. Trehalose enhanced fluidity of the goat sperm membrane and its protection during freezing. *Biol Reprod* 2003; **69**(4): 1245-1250.

[21] Medeiros CMO, Forell F, Oliveira ATD, Rodrigues JL. Current status of sperm cryopreservation: Why isn't it better? *Theriogenology* 2002; **57**(1): 327-344.

[22] Patel HA, Siddiquee GM, Chaudhari DV, Suthar VS. Effect of different antioxidant additives in semen diluent on cryopreservability (-196 ℃) of buffalo semen. *Vet World* 2016; **9**(3): 299-303.

[23] Rastegarnia A, Shahverdi A, Topraggaleh TR, Ebrahimi B, Shafipour V. Effect of different thawing rates on post-thaw viability, kinematic parameters and chromatin structure of buffalo (*Bubalus bubalis*) spermatozoa. *Cell J* 2013; **14**(4): 306-313.

[24] Nair SJ, Brar AS, Ahuja CS, Sangha SPS, Chaudhary KC. A comparative

study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. *Anim Reprod Sci* 2006; **96**(1-2): 21-29.

[25]Kadirvel G, Kumar S, Kumaresan A. Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. *Anim Reprod Sci* 2009; **114**(13): 125-134.

[26]Strzezek J. Secretary activity of boar seminal vesicle glands. *Reprod Biol* 2002; **2**(3): 243-266.

[27]Ahmad E, Aksoy M. Trehalose as a cryoprotective agent for the sperm cells: A mini review. *Anim Health Prod Hyg* 2012; **1**(2): 123-129.

[28]Badr MR, Mary GA, Hassan MH. Effect of trehalose on cryopreservation, oxidative stress and DNA integrity of buffalo spermatozoa. *J Reprod Infertil* 2010; **1**(2): 50-57.

[29]Shahba MI, El-Sheshtawy RI, El-Azab AI. Improvement of buffalo semen freezability by using TRIS extender enriched with different concentrations of trehalose/sucrose. *J Innov Pharm Biol Sci* 2016; **3**(1): 198-203.

[30]Ahmad E, Naseer Z, Ahmad N. High trehalose concentration in tris-based egg yolk extender has detrimental effect on post-thaw semen quality in Nili-Ravi buffalo bull. *Anim Health Prod Hyg* 2013; **2**(2): 213-215.

[31]Bucak MN, Tuncer PB, Sarıözkan S, Ba pınar N, Ta pınar M, Coyan K, et al. Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: Antioxidants protect DNA integrity against cryodamage. *Cryobiology* 2010; **61**(3): 248-253.

[32]Pena AI, Barrio, F, Quintela, LA, Herradon PG. Proline and glycine betaine in a diluent for freezing canine spermatozoa. *Reprod Domest Anim* 1998; **33**(1): 5-9.

[33]Uysal O, Bucak MN. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Vet Brno* 2007; **76**(3): 383-390.

[34]Ansari MS, Rakha BA, Akhter S. Effect of L-cysteine in extender on post-thaw quality of Sahiwal bull semen. *Anim Reprod Sci* 2011; **29**(3): 197-203.

[35]Guerin P, Guillaud J, Menezo Y. Hypotaurine in spermatozoa and genetal secretions and its production by oviduct epithelial cells *in vitro*. *Hum Reprod* 1995; **10**(4): 866-872.

[36]Badr MR, Azab AMS, Rawash ZM. Effect of trehalose, cysteine and hypotaurine on buffalo bull sperm freezability, ultrastructure changes and fertilizing potentials. *Assiut Vet Med J* 2014; **60**(142): 38-45.

[37]Longobardi V, Salzano A, Campanile G, Marrone R, Palumbo F, Vitiello M, et al. Carnitine supplementation decreases capacitation-like changes of frozen-thawed buffalo spermatozoa. *Theriogenology* 2017; **88**: 236-243.

[38]Parikh S, Saneto R, Falk MJ, Anselm I, Cohen BH, Haas R. A modern approach to the treatment of mitochondrial disease. *Curr Treat Options Neurol* 2009; **11**(6): 414-430.

[39]Arduini A. Carnitine and its acyl esters as secondary antioxidants? *Am Heart J* 1992; **123**(6): 1726-1727.

[40]Mohamed EL-Raey, Badr MR, Assi MM, Rawash ZM. *L*-carnitine enhancing roles on buffalo semen freezability, ultra structure and fertilizing potentials. *Assiut Vet Med J* 2016; **62**(149): 163-173.

[41]Abd El-Razek IM, El-Shamaa IS. Effect of glutamine addition to freezing extender on crypreserved semen parameters and subsequent fertility of Egyptian buffalo bull. *Asian J Anim Vet Adv* 2016; **11**(5): 296-302.

[42]Amirat-Briand L, Bencharif D, Vera-Munoz O, Ali HBH, Destrumelle S, Desherces S, et al. Effect of glutamine on post-thaw motility of bull spermatozoa after association with LDL (low density lipoproteins) extender: Preliminary results. *Theriogenology* 2009; **71**(8): 1209-1214.

[43]Reiter RJ. The pineal gland: Reproductive interactions. In: Pang PKT, Schreibman MP, editors. *Vertebrate endocrinology: Fundamental and biomedical implications*. San Diego, US: Academic Press Inc.; 1991, p. 269-310.

[44]Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter R. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J Pineal Res* 2007; **42**(1): 28-42.

[45]Kang JT, Koo OJ, Kwon DK, Park HJ, Jang G, Kang SK, et al. Effects of melatonin on *in vitro* maturation of porcine oocyte and expression of melatonin receptor RNA in cumulus and granulosa cells. *J Pineal Res* 2009; **46**(1): 22-28.

[46]Casao A, Mendoza N, Pérez-Pé R, Grasa P, Abecia JA, Forcada F, et al. Melatonin prevents capacitation and apoptotic-like changes of ram spermatozoa and increases fertility rate. *J Pineal Res* 2010; **48**(1): 39-46.

[47]El-Raey M, Badr MR, Rawash ZM, Darwish GM. Evidences for the role of melatonin as a protective additive during buffalo semen freezing. *Am J Anim Vet Sci* 2014; **9**(4): 252-262.

[48]Mohamed EL-Raey, Badr MR, Assi M, Rawash ZM. Effect of melatonin on buffalo bull sperm freezability, ultrastructure changes and fertilizing potentials. *Assiut Vet Med J* 2015; **61**(144): 201-208.

[49]Molpeceres V, Mauriz JL, García-Mediavilla MV, González P, Barrio JP, González-Gallego J. Melatonin is able to reduce the apoptotic liver changes induced by aging via inhibition of the intrinsic pathway of apoptosis. *J Gerontol Biol Med Sci* 2007; **62**(7): 687-695.

[50]Sanchez-Gutierrez M, Garcıa-Montalvo EA, Izquierdo-Vega JA, Del Razo LM. Effect of dietary selenium deficiency on the *in vitro* fertilizing ability of mice spermatozoa. *Cell Biol Toxicol* 2008; **24**(4): 321-329.

[51]Zubair M, Ali M, Ahmad M, Sajid SM, Ahmad I, Gul ST. Effect of Selenium and vitamin E on cryopreservation of semen and reproductive performance of animals. *J Entomol Zool Studies* 2015; **3**(1): 82-86.

[52]Dorostkar K, Alavi-Shoushtari SM, Mokarizadeh A. Effects of *in vitro* selenium addition to the semen extender on the spermatozoa characteristics before and after freezing in water buffaloes (*Bubalus bubalis*). *Vet Res Forum* 2012; **3**(4): 263-268.

[53]Badr MR, Ziada MS, Darwish GM, Nasra AA. Influence of antioxidants on freezability, *in vitro* fertilizing potential and conception rate of buffalo spermatozoa. *Assiut Vet Med J* 2003; **49**(99): 261-279.

[54]Borghese A. Development and perspective of buffalo and buffalo market in Europe and Near East. *Rev Vet* 2010; **21**(1): 1-18.

[55]Chaikhun T, Tharasanit T, Rattanatep J, de Rensis F, Techakumphu M. Fertility of swamp buffalo following the synchronization of ovulation by the sequential administration of GnRH and PGF_2alpha combined with fixed-timed artificial insemination. *Theriogenology* 2010; **74**(8): 1371-1376.

[56]Ravinder R, Kaipa O, Baddela VS, Singhal Sinha E, Singh P, Nayan V, et al. Saliva ferning, an unorthodox oestrus detection method in Water Buffaloes (*Bubalus bubalis*). *Theriogenology* 2016; **86**(5): 1147-1155.

[57]Campanile G, Baruselli PS, Neglia G, Vecchio D, Gasparrini B, Gimenes

LU, et al. Ovarian function in the buffalo and implications for embryo development and assisted reproduction. *Anim Reprod Sci* 2010; **121**(1-2): 1-11.

[58]Chasombat J, Nagai T, Parnpai R, Vongpralub T. Ovarian follicular dynamics and hormones throughout the estrous cycle in Thai native (*Bos indicus*) heifers. *Anim Sci J* 2014; **85**(1): 15-24.

[59]Porto-Filho RM, Gimenes LU, Monteiro BM, Carvalho NAT, Ghuman SPS, Madureira EH, et al. Detection of estrous behavior in buffalo heifers by radiotelemetry following $PGF_2\alpha$ administration during the early or late luteal phase. *Anim Reprod Sci* 2014; **144**(3-4): 90-94.

[60]Kumar PR, Singh SK, Kharche SD, Govindaraju CS, Behera BK, Shukla SN, et al. Anestrus in cattle and buffalo. *Indian Perspect Adv Anim Vet Sci* 2014; **2**(3): 124-138.

[61]Perera BM. Reproductive cycles of buffalo. *Anim Reprod Sci* 2011; **124**(3-4): 194-199.

[62]Jha SS. Ovarian responses of buffalo treated with PGF_2 alpha. *Asian J Biochem Pharm Res* 2011; **1**(4): 377-379.

[63]Mirmahmoudi R, Prakash BS. The endocrine changes, the timing of ovulation and the efficacy of the Doublesynch protocol in the Mur-rah buffalo (*Bubalus bubalis*). *Gen Comp Endocr* 2012; **177**(1): 153-159.

[64]Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. *Theriogenology* 1995; **44**(7): 915-923.

[65]Baruselli PS, Carvalho NAT, Gimenes LU, Crepaldi GA. Fixed-time artificial insemination in buffalo. *Ital J Anim Sci* 2007; **6**(2): 107-118.

[66]Neglia G, Restucci B, Russo M, Vecchio D, Gasparrini B, Prandi A, et al. Early development and function of the corpus luteum and relationship to pregnancy in the buffalo. *Theriogenology* 2015; **83**(6): 959-967.

[67]Campanile G, Neglia G, D'Occhio MJ. Embryonic and fetal mortality in river buffalo (*Bubalus bubalis*). *Theriogenology* 2016; **86**(1): 207-213.

[68]Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim Reprod Sci* 2004; **82–83**: 479-486.

[69]Karen AM, Darwish SA. Efficacy of Ovsynch protocol in cyclic and acyclic Egyptian buffaloes in summer. *Anim Reprod Sci* 2010; **119**(1-2): 17-23.

[70]Negli G, Gasparrini B, Salzano A, Vecchio D, de Carlo E, Cimmino R, et al. Relationship between the ovarian follicular response at the start of an Ovsynch–TAI program and pregnancy outcome in the Mediterranean river buffalo. *Theriogenology* 2016; **86**(9): 2328-2333.

[71]Khumran AM, Roshina Y, Ariff MO, Wahid H, Dhaliwala G, Khanh NP, et al. Comparision of estrus response and pregnancy rate of beef cows synchronized with progesterone and prostaglandin based protocols. *J Adv Vet Anim Res* 2012; **11**(1): 3561-3567.

[72]Monteiro BM, de Souza DC, Vasconcellos GSFM, Correa TB, Vecchio D, de Sá Filho MF, et al. Ovarian responses of dairy buffalo cows to timed artificial insemination protocol, using new or used progesterone devices, during the breeding season (autumn–winter). *J Anim Sci* 2016; **87**(1): 13-20.

[73]Kundulkar AD, Ingawale MV, Deshmukh SG, Hajare SW, Ingole RS. Absence of potential benefit of progesterone priming during ovsynch protocol in postpartum anestrous buffalo near the end of breeding season. *Indian J Anim Reprod* 2016; **37**(2): 61-62.

[74]Cirit U, Ak K, Ileri IK. New strategies to improve the efficiency of the Ovsynch protocol in primiparous dairy cows. *Bull Vet Instit Pulawy* 2007; **51**(1): 47-51.

[75]Dhindsa SS, Honparkhe M, Dadarwal D, Grewal RS, Singh M, Brar PS. Economic impact of doublesynch protocol in buffaloes during summer season. *Int J Sci Environ Technol* 2016; **5**(5): 2907-2911.

[76]Mirmahmoudi R, Prakash BS. The endocrine changes, the timing of ovulation and the efficacy of the Doublesynch protocol in the Murrah buffalo (*Bubalus bubalis*). *Gen Comp Endocr* 2012; **177**(1): 153-159.

[77]Ramadan TA, Sharma RK, Phulia SK, Balhara AK, Ghuman SS, Singh I. Manipulation of reproductive performance of lactating buffaloes using melatonin and controlled internal drug release device treatment during out-of-breeding season under tropical conditions. *Theriogenology* 2016; **86**(4): 1048-1053.

[78]Yousuf MR, Martins JP, Husnain A, Riaz U, Riaz H, Sattar A, et al. Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo. *Anim Reprod Sci* 2015; **159**: 104-108.

[79]Mirmahmoudi R, Souri M, Prakash BS. Comparison of endocrine changes, timing of ovulations, ovarian follicular growth, and efficacy associated with Estradoublesynch and Heatsynch protocols in Murrah buffalo cows (*Bubalus bubalis*). *Theriogenology* 2014; **82**(7): 1012-1020.

[80]Jacomini JO, Macedo GG, Carvalho NAT, Sales JN, Baruselli PS. LH surge in response to the treatment with GnRH analog or estradiol in ovariectomized buffaloes with or without progesterone pre-exposition. *Livestock Sci* 2014; **160**: 194-198.

[81]de Carvalho NA, Soares JG, Baruselli PS. Strategies to overcome seasonal anestrus in water buffalo. *Theriogenology* 2016; **86**(1): 200-206.

[82]Carvalho NAT, Soares JG, Souza DC, Vannucci FS, Amaral R, Maio JRG, et al. Different circulating progesterone concentrations during synchronization of ovulation protocol did not affect ovarian follicular and pregnancy responses in seasonal anestrous buffalo cows. *Theriogenology* 2014; **81**(3): 490-495.

[83]Haider MS, Hassan M, Khan AS, Husnain A, Bilal M, Pursley JR, et al. Effect of timing of insemination after CIDR removal with or without GnRH on pregnancy rates in Nili-Ravi buffalo. *Anim Reprod Sci* 2015; **163**: 24-29.

[84]Lu YQ, Zhang M, Lu S, Xu D, Huang W, Meng B, et al. Sex-preselected buffalo (*Bubalus bubalis*) calves derived from artificial insemination with sexed sperm. *Anim Reprod Sci* 2010; **119**(3-4): 169-171.

[85]Presicce GA, Verberckmoes S, Senatore EM, Klinc P, Rath D. First established pregnancies in Mediterranean Italian buffaloes (*Bubalus bubalis*) following deposition of sexed spermatozoa near the utero tubal junction. *Reprod Dom Anim* 2005; **40**(1): 73-75.

[86]Campanile G, Gasparrini B, Vecchio D, Neglia G, Senatore EM, Bella A, et al. Pregnancy rates following AI with sexed semen in Mediterranean Italian buffalo heifers (*Bubalus bubalis*). *Theriogenology* 2011; **76**(3): 500-506.

[87]Lu YQ, Liao YQ, Zhang M, Yang BZ, Liang XW, Yang XG, et al. A field study on artificial insemination of swamp and crossbred buffaloes with sexed semen from river buffaloes. *Theriogenology* 2015; **84**(6): 862-867.

[88]Cerchiaro I, Cassandro M, Dal Zotto R, Carnier P, Gallo L. A field study on fertility and purity of sex-sorted cattle sperm. *J Dairy Sci* 2007; **90**(5): 2538-2542.

[89]DeJarnette JM, Nebel RL, Marshall CE, Moreno JF, McCleary CR, Lenz

RW. Effect of sex-sorted sperm dosage on conception rates in Holstein heifers and lactating cows. *J Dairy Sci* 2008; **91**(5): 1778-1785.

[90]Norman HD, Hutchison JL, Miller RH. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. *J Dairy Sci* 2010; **93**(8): 3880-3890.

[91]de Araujo Berber RC, Madureira EH, Baruselli PS. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (*Bubalus bubalis*). *Theriogenology* 2002; **57**(5): 1421-1430.

[92]Verberckmoes S, Van Soom A, De Pauw I, Dewulf J, Vervaet C, de Kruif A. Assessment of a new utero-tubal junction insemination device in dairy cattle. *Theriogenology* 2004; **61**(1): 103-115.

[93]Verberckmoes S, Dewulf J, De Pauw I, Van Soom A, de Kruif A. Assessment of a new utero-tubal junction insemination device in dairy cattle under field conditions. Proceedings of the 10th International Symposium on Veterinary Epidemiology and Economics; 17-21 November 2003; Vina del Mar, Chile.

[94]FAO. *FAOSTAT database on population*. Rome, Italy: FAO; 2014.

[95]Sethi RK. Improving riverine and swamp buffaloes through breeding. Proceedings of the 4th Asian Buffalo Congress on Buffalo for Food Security and Rural Employment. New Delhi, India; 2003, vol 1, p. 50-61.

[96]Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* 1998; **280**(5367): 1256-1258.

[97]Seidel, GE Jr. Brief introduction to whole genome selection in cattle using single nucleotide polymorphisms. *Reprod Fertil Dev* 2010; **22**(1): 138-144.

[98]Jonathan M. Schefers and Kent A. Weigel. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. *Anim Front* 2014; **2**(1): 4-9.

[99]Alexiev A, Vlakhov K, Karaivanov C, Kacheva D, Palikhronov O, Petrov M, et al. Embryo transfer in buffaloes in Bulgaria. Proceedings of the 2nd World Buffalo Congress; 12-16 December 1988; New Delhi, India; 1988, p. 591-595.

[100]Senatore EM, De Santis G, Barile V. Corpus luteum measurements, echotexture and plasma progesterone in adult and puberal Mediterranean Italian buffalo (*Bubalus bubalis*). *Theriogenology* 2002; **57**(1): 792.

[101]Mann GE. Corpus luteum size and plasma progesterone concentration in cows. *Anim Reprod Sci* 2009; **115**(1-4): 296-269.

[102]Tian YQ, Zhao XX. Ovarian follicle activity in yak versus cattle and buffalo. In: Zhao XX, Zhang CR, editors. *Recent advances in yak reproduction*. New York, USA: International Veterinary Information Service; 2000.

[103]Taneja, M, Totey SM, Ah A. Seasonal variation in follicular dynamics of superovulated Indian water buffalo. *Theriogenology* 1995; **43**(2): 45l-464.

[104]Palta P, Bansal N, Prakash BS, Manik RS, Madan ML. Follicular fluid inhibin concentrations in relation to follicular diameter and estradiol-17 β . Progesterone and testosterone concentrations in individual buffalo ovarian follicles. *Indian J Exp Biol* 1998; **36**: 768-774.

[105]Afify AA, Kassim Nany SI, Hassan Hoda Z, Zeidan Soheir M. Relationship between photoperiod and melatonin level at growth stages of buffalo heifers. *Egyptian J Basic Appl Physiol* 2008; **2**(7): 473-450.

[106]Parmeggiani A, Seren E, Esposito L, Borghese A, Di Palo R, Terzano GM. *Plasma levels of melatonin in buffalo cows.* Proceedings of International Symposium Prospect of Buffalo Production in Mediterranean and the Middle East; 9-12 November 1992; Cairo, Egypt. Wageningen, Netherlands: Pudoc Scintific Publischer; 1993, vol 62, p. 401-403.

[107]Gamit VV, Rao TK, Parikh SS, Gamit PM. Advanced heat detection aids and its applications in dairy farm. *Sch J Agric Vet Sci* 2015; **2**(4A): 287-294.

[108]Kumar PR, Singh SK, Kharche SD, Govindaraju CS, Behera BK, Shukla SN, et al. Anestrus in cattle and buffalo: Indian perspective. *Adv Anim Vet Sci* 2014; **2**(3): 124-138.

[109]Lopez H, Satter LD, Wiltbank MC. Relation between level of milk production and estrus behavior of lactating dairy cow. *Anim Reprod Sci* 2004; **81**(3): 209-223.

[110]Singh B, Dixit VD, Singh P, Georgie GC, Dixit VP. Plasma inhibin levels in relation to steroids and gonadotrophins during the oestrus cycle in buffalo. *Reprod Domest Anim* 2001; **36**(3-4): 163-167.

[111]Findlay JK. Peripheral and local regulators of folliculogenesis. *Reprod Fertil Dev*1994; **6**(2): 127-139.

[112]Ronchi B, Stradaioli G, Verini Supplizi A, Bernabucci U, Lacetera N, Accorsi P. Influence of heat stress or feed restriction on plasma P4, oestradiol 17 β , LH, FSH, prolactin and cortisol in Holstein heifers. *Livest Prod Sci* 2001; **68**(2-3): 231-241.

[113]Phogat JB, Pandey AK, Singh I. Seasonality in buffaloes reproduction. *Int J Plant Anim Environ Sci* 2016; **6**(2): 46-54.

[114]Ismail S T, Abdoun MY, Tawfik M S, Mohammed KM, Shawki H. Effect of PMSG and FSH on the ovarian response and embryo production in buffaloes. The 4th Annual Congress of Egyptian Society, Animal Reproduction and Fertility; 1992.

[115]Mohammed KME. Studies on embryo transfer in buffalo and Friesian cows [Thesis]. Cairo, Egypt: Faculty Veterinary Medicine, Cairo University; 1995.

[116]Bó GA, Mapletoft RJ. Historical perspectives and recent research on superovulation in cattle. *Theriogenology* 2014; **81**(1): 38-48.

[117]Sumretprasong J, Leangcharuen N, Thuangsanthia A, Thijae K. Dose response to superovulation in Thai dairy cattle. Proceedings of the 15th Congress of FAVA; 27-30 October 2008; Bangkok, Thailand; 2008, p. 247-248.

[118]Koji Kimura. Superovulation with a single administration of FSH in aluminum hydroxide gel: A novel superovulation method for cattle. *J Reprod Dev* 2016; **62**(5): 423-429.

[119]Madan ML. Embryo transfer technology in buffaloes. *Indian J Anim Reprod* 1992; **13**: 108-117.

[120]Techakumphu M, Sukavong Y, Intaramongko S, Intaramongkol J. The effect of gonadotropin releasing hormone on superovulation in elite swamp buffalo cows (*Bubalus bubalis*). *J Vet Med Sci* 2001; **63**(8): 853-857.

[121]Singh N, Dhaliwal GS, Malik VS, Dadarwal D, Honparkhe M, Singhal S, et al. Comparison of follicular dynamics, superovulatory response, and embryo recovery between estradiol based and conventional superstimulation protocol in buffaloes (*Bubalus bubalis*). *Vet World* 2015; **8**(8): 983-988.

[122]Uoc NT, Nguyen BX, Ty LV, Long DD, Chupin D, Becker F, et al. Superovulation in swamp buffalo: Effect of estradiol supplementation of gonadotrophin treatment. *Theriogenology* 1992; **38**(3): 471-478.

[123]Singh M, Matharoo JS. Studies of some factors affecting embryo

transfer in buffalo. Proceedings of the Third World Buffalo Congress; 13-17 May 1991; Varna, Bulgaria; 1991, p. 88.

[124]Mikkola M. Superovulation and embryo transfer in dairy cattle– effect of management factors with emphasis on sex-sorted semen [Thesis]. Helsinki, Finland: Faculty of Veterinary Medicine, University of Helsinki, Finland, 2017.

[125]Tríbulo A, Rogan D, Tribulo H, Tribulo R, Alasino RV, Beltramo D, et al. Superstimulation of ovarian follicular development in beef cattle with a single intramuscular injection of Folltropin-V. *Anim Reprod Sci* 2011; **129**(1-2): 7-13.

[126]Kimura K, Hirako M, Iwata H, Aoki M, Kawaguchi M, Seki M. Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel. *Theriogenology* 2007; **68**(4): 633-639.

[127]Karaivanov C, Vlahov K, Petrov M, Kacheva D, Aleksiev A, Polihronov O. Superovulation in buffaloes with FSH. *Vet Med Sci* 1987; **24**: 57-63.

[128]Cliff Lamb. Factors affecting an embryo transfer program. Proceedings of Applied Reproductive Strategies in Beef Cattle; 27-28 October 2005; Reno, Nevada.

[129]Sales JN, Iguma LT, Batista RI, Quintao CC, Gama MA, Freitas C, et al. Effects of a high energy diet on oocyte quality and *in vitro* embryo production in *Bos indicus* and *Bos taurus* cows. *J Dairy Sci* 2015; **98**(5): 3086-3099.

[130]Durlinger A, Visser J, Themmen A. Regulation of ovarian function: the role of anti-Mullerian hormone. *Reproduction* 2002; **124**(5): 601-609.

[131]Rico C, Drouilhet L, Salvetti P, Dalbiès-Tran R, Jarrier P, Touze JL, et al. 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. *Reprod Fertil Dev* 2012; **24**(7): 932-944.

[132]Souza AH, Verstegen JP, Batista E, Collar C, Baruselli PS, Wiltbank MC. Using information on circulating levels of Anti-Mullerian hormone (AMH) to enhance embryo production and fertility in cattle. Proceedings of the American Embryo Transfer Association (AETA); 12-15 September 2014; Middleton, Wisconsin. Champaign, IL: AETA; 2014.

[133]Liang A, Salzano A, D'Esposito M, Comin A, Montillo M, Yang L, et al. Anti-Mullerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor type][and LH receptor in granulosa cells as predictive markers of good buffalo (*Bubalus bubalis*) donors. *Theriogenology* 2016; **86**(4): 963-970.

[134]Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JLH, et al. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod Fertil Dev* 2010; **23**(1): 1-14.

[135]Barkawi AH, Hafez YM, Ibrahim SA, Ashour G, El-Asheeri AK, Ghanem N. Characteristics of ovarian follicular dynamics throughout the estrous cycle of Egyptian buffaloes. *Anim Reprod Sci* 2009; **110**(3-4): 326-334.

[136]Misra AK, MuthaRao M, Kasiraj MR, Ranga Reddy NS, Pant HC. Factors affecting pregnancy rate following nonsurgical embryo transfer in buffalo (*Bubalus bubalis*): A retrospective study. *Theiogenology* 1999; **52**(1): 1-10.

[137]Soliman SS, Attia MZ, Abdoon AS, Sayed El-Toukhey NS, Kandil OM, Sabra HA. Seasonal variation in ovarian functions in Egyptian buffalo and cattle. *Int J Pharm Tech Res* 2016; **9**(6): 34-42.

[138]Abdoon ASS, Gabler C, Holder C, Kandil OM, Einspanier R. Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (*Bubalus bubalis*) oocytes. *Theriogenology* 2014; **82**(8): 1055-1067.

[139]Dash S, Chakravarty AK, Singh A, Upadhyay A, Singh M, Yousuf S. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Vet World* 2016; **9**(3): 235-244.

[140]Heleil B, El-Deeb Y. Superovulatory response in relation to follicular dynamics and presence of dominant follicles in Egyptian buffaloes. *Adv Biol Res* 2010; **4**(3): 169-174.

[141]Aerts JM, Bols PE. Ovarian follicular dynamics. A review with emphasis on the bovine species. Part][: Antral development, exogenous influence, and future prospects. *Reprod Domest Anim* 2008; **45**(1): 180-187.

[142]Blondin P, Vigneault C, Nivet AL, Sirard MA. Improving oocyte quality in cows and heifers-What have we learned so far? *Anim Reprod* 2012; **9**(3): 281-289.

[143]Spell AR, Beal WE, Corah LR, Lamb GC. Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. *Theriogenology* 2001; **56**: 287-298.

[144]Misra AK, JoshI BV. Relationship between the synchrony of donor-recipient oestrus in successful pregnancies of embryo transfer in buffalo. *Buffalo J* 1991; **7**(1): 71-75.

[145]Misra AK, MuthaRao M, Kasiraj MR, Ranga Reddy NS, Pant HC. Factors affecting pregnancy rate following nonsurgical embryo transfer in buffalo (*Bubalus bubalis*): A retrospective study. *Theiogenology* 1999; **52**(1): 1-10.

[146]Dobrinsky JR. Advancements in cryopreservation of domestic animal embryos. *Theriogenology* 2002; **57**(1): 285-302.

[147]Mohammed KM, Ismail ST, Rezk Y, Tawfik MS. Factors affecting viability of deep frozen buffalo-embryos. *Egypt J Agric Res* 2000; **78**(2): 877-892.

[148]Kasiraj R, Rao MM, Rangareddy NS, Misra AK. Superovulatory response in buffaloes following a single subcutaneous or multiple intramuscular FSH administration. *Theriogenology* 1992; **37**(1): 234.

[149]Niemann H. Cryopreservation of ova and embryos from livestock: current status and research needs. *Theriogenology* 1991; **35**(1): 109-123.

[150]Caamaño JN, Gómez E, Trigal B, Muñoz M, Carrocera S, Martín D, Díez C. Survival of vitrified *in vitro*-produced bovine embryos after a one-step warming in-straw cryoprotectant dilution procedure. *Theriogenology* 2015; **83**(5): 881-890.

[151]Mahmoud KM, Scholkamy TH, Darwish SF. Improvement of vitrification of *in vitro* produced buffalo embryos with special reference to sex ratio following vitrification. *Iran J Vet Res* 2015; **16**(4): 325-330.

[152]Van Waglendonk-de Leeuw AM, den Daas JHG, Rail WF. Field trial to compare pregnancy rates of bovine embryo cryopreservation methods: Vitrification and one-step dilution verses slow freezing and three-step dilution. *Theriogenology* 1997; **48**: 1071.

[153]DADIS. *Domestic animal diversity information system*. Rome: Food and Agriculture Organization (FAO); 2011.

[154]Nigm A. Buffalo recording and breeding practices in Egypt: A case study. Proceedings of the Workshop on Animal Recording for Improved Breeding and Management Strategies for Buffaloes. 16-17 May 2000; Bled, Slovenia; 2000, p. 33-36.