Applications of Assisted Reproduction

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Basics

- ***Assisted reproduction:*** An enabling technology that allows the livestock industries to achieve faster rates of genetic gain by making greater use of individual males and females with commercially important traits (Barucelli et al, 2023)
- ***Includes:*** Synchronization of the time of breeding, influencing the age at first breeding, the interval between the calving and the first breeding, and breeding during seasonal anestrus
- ***Pathway:*** Reproductive biology >>> fundamental discoveries >>> assisted reproduction
- ***Example:*** Folliculogenesis + oocyte maturation + early embryonic development >>> OPU + IVF
- ***Species-specific:*** Development in once species, adopted in another and refined to be specific
- ***Buffaloes:*** Artificial Insemination through cloning: Cattle >>> sheep >>> goats buffaloes

AI and Semen preservation and seasonal breeding

- The first buffalo calf born to artificial insemination (AI) was produced by Bhattacharya and colleagues in 1943.
- It has been assumed that egg yolk-based semen extenders, which were undergoing development in cattle, were also used in buffaloes.
- Widespread adoption of AI only became possible with sperm cryopreservation.
- Buffalo sperm are more susceptible to damage when frozen-thawed; conditions have been refined for optimizing sperm cryopreservation in buffaloes.
- Artificial insemination has made possible improvements in the quality of buffalo milk in Asia, Europe, and the Americas.
- Buffaloes calving during the normal breeding season (NBS, August to January) showed a postpartum estrus interval of 55.95 days versus 91.15 days in those calving during the low breeding season (LBS, February to July). MPL in the LBS remained lower than the NBS ($p<0.01$) (Qureshi et al, 1999).
- It was concluded that onset of breeding season was associated with increasing metabolizable energy intake (MEI) and decreasing crude protein and minerals intake.

Synchronization of estrus and fixed-time AI

- Artificial insemination in buffaloes initially relied on the detection of estrus (Vale et al., 1994; Baruselli, 1994).
- However, estrous detection is problematic in buffaloes because of the low intensity of estrus and the wide variation in estrous duration (4–64 hours).
- Combinations of GnRH, estradiol, progestogens, and PGF2a can be used to control ovarian follicular waves and the time of ovulation in buffaloes.
- Equine gonadotropin (eCG) can be added at the end of a synchronization protocol to facilitate the final stages of follicle/oocyte development, tighten the time of ovulation, and increase fertility mainly in anestrous buffalo.
- Pregnancy rates are lower when estrus synchronization + FTAI are used in the non-breeding season in buffaloes synchronized with the GnRH + PGF2a + GnRH-based protocol Ovsynch.
- Higher incidence of anestrous and relatively high embryonic mortality were observed in buffaloes bred during the non-breeding season.

Embryo transfer and Superovulation

- Embryo transfer from donor females to recipients allows individual female buffaloes with commercially important traits to make a greater contribution to genetic improvement.
- The first successful embryo transfer in buffaloes involved the non-surgical collection of a single 7-day blastocyst from a donor and non-surgical transfer to a recipient.
- The power of embryo transfer is greatly increased by the collection of multiple embryos from elite donor females.
- This requires ovarian follicular supertimulation, commonly referred to as superovulation.
- Early attempts at superovulation in buffaloes drew from experiences in cattle with FSH, PMSG and GnRH in combination with PGF2a.
- Ultrasound monitoring during superovulation in buffaloes shows the growth on average of around 10 follicles (>8 mm).
- The lower embryo recovery in superovulated buffaloes has restricted the widespread adoption of this assisted reproduction technology in buffaloes.

In vitro fertilization

- The failure of superovulation as a viable assisted reproduction technology to exploit female genetics in buffaloes has directed focus to in vitro fertilization (IVF).
- Oocyte pickup (OPU) and in vitro embryo production (IVEP) are seen as important assisted reproduction technologies for producing a relatively large number of embryos in buffaloes.
- The present major constraint of OPU/IVEP in buffaloes is the relatively small number of oocytes that can be recovered from donors, due to the small follicular reserve in buffaloes and modest response to follicular superstimulation treatment prior to OPU.
- Another limiting factor is seasonality in buffaloes that is associated with reduced oocyte competence during the non-breeding season.
- Refinement of IVEP procedures has led to relatively high rates of blastocyst development in buffaloes.
- Variation among bulls to cryopreservation damage remains an unresolved constraint in buffalo IVEP.

Blastocyst/embryo culture

- Buffalo embryos were first cultured in vivo in an intermediate host such as the ligated oviducts of sheep.
- This was followed by cell co-culture systems and then defined media including synthetic oviduct fluid and potassium simplex optimized medium.
- Buffalo oocytes commonly undergo IVM in TCM199 supplemented with serum and hormones including gonadotropins and 17b-estradiol.
- Major increases in blastocyst yield in buffaloes have resulted from improvements in IVM whereas advances in IVC have had a lesser impact.
- The refreshment of IVC medium to remove reactive oxygen species, ammonia, and waste products of metabolism does not impact embryo development in buffaloes as occurs for other species.
- Buffalo embryos require relatively high concentrations (1.5 mM) of glucose during early embryonic development whereas sheep and cattle embryos show increased glucose consumption during late culture when compaction occurs.

Oocyte Pick Up (OPU)

- Oocyte pick up OPU combined with IVEP allows greater use in genetic improvement programs of female buffaloes with high genetic merit.
- The application of OPU in buffaloes is somewhat restricted because of the low number of ovarian follicles and viable oocytes recovered and a seasonal effect on oocyte quality.
- Buffaloes show large individual variation in the number of antral follicles leading to variation in the number of oocytes recovered by OPU (range of 0–30).
- Circulating concentrations of Anti-Mullerian Hormone provide an endocrine marker of the AFs and can be used to screen potential oocyte donors.
- A greater number of embryos are produced by IVEP during short days which coincides with the breeding season at higher latitudes.
- Follicular superstimulation with FSH before OPU increases the number of medium and large follicles in buffalo heifers, and primiparous and multiparous cows. This is associated with a greater number of viable oocytes available for OPU-IVEP which further optimizes the efficiency of this assisted reproduction in buffaloes.

Juvenile in vitro embryo transfer

- Juvenile in vitro embryo transfer (JIVET) is an assisted reproduction technology that provides the opportunity to significantly reduce generation intervals and accelerate genetic improvement.
- It involves the recovery of oocytes from prepubertal buffalo heifers combined with IVF to produce transferable embryos.
- The ovaries of prepubertal buffalo heifers were reported to have 10 000–15 000 primordial follicles.
- Ovarian follicular waves are established in prepubertal heifers, and follicles are responsive to follicular supertimulation treatments.
- It is possible to stimulate follicular growth in prepubertal buffalo heifers and recover oocytes for IVF.
- In calves, oocytes are recovered using laparoscopic ovum pick up (LOPU).
- The first reports of JIVET births in buffaloes were in 2017/2018.

Cloning

- The first live cloned buffalo calves resulted from established somatic cell nuclear transfer using fetal fibroblasts and granulosa cells.
- Cloning has been adopted in India to clone bulls with elite genetics.
- The cloned bulls are used to obtain semen that is cryopreserved and distributed for use in national buffalo genetic improvement programs.
- This should accelerate the genetic improvement of buffaloes in India and is a good example of the impact assisted reproduction can have on livestock production resulting in economic and social benefits.
- Notwithstanding the application in India, technical challenges and inefficiencies have limited the adoption of cloning for genetic improvement in buffaloes.
- The emergence of CRISPR-Cas9 technology has provided the opportunity to achieve targeted genetic change in livestock including buffaloes.
- CRISPR-Cas9 technology enables geneticists and medical researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence.