There are about 151.5 million water buffaloes in the world, 96.6% of which are found in Asia. Genetic improvement of this important animal resource for milk and meat is limited by inherent biological attributes such as long generation interval and low reproductive rate. However, developments in reproductive biotechniques in other species offer great possibilities for achieving the desired genetic improvement in water buffaloes. Artificial insemination using frozen semen and manipulation of ovarian function for estrus synchronization are now common. Superovulatory treatments in females to produce embryos in vivo yielded only an average of transferable embryos of 1.8 - 2.1 per collection. Production of embryos in vivo using ovum pick-up (OPU) in combination with IVM/IVF has been tried to a limited extent, and may become an alternative to superovulation and in vivo embryo production in buffaloes.
Techniques developed in vitro maturation and in vitro fertilization (IVM/IVF) in cattle offer another way of producing buffalo embryos. Attempts to apply these as part of the buffalo embryo transfer is a recent development. Indeed, the first in vitro-derived embryo calf was born only in 1991 (Madan et al. 1991), eight years after the first in vivo-derived embryo calf was produced.

Another interesting technique so far employed along this area is aspiration of immature oocytes from live animals, raising the possibility of producing more offspring from very superior donors.

Discussion of other related biotechniques such as nuclear transfer and gene transfer will not be covered in this paper, since there is no substantial work done using these techniques in buffaloes.

REPRODUCTIVE BIOTECHNIQUES USED IN WATER BUFFALOES

AI and Related Techniques

The earliest development in farm animal reproduction technology was the use of AI. This technique was used for water buffalo in the early forties at the Allahabad Agricultural Institute in India. The first calf was born in 1943 (Ranhan and Pathar 1993).

Following the successful report of Polge and Rowson (1952) on freezing cattle semen at ultra low temperature (-79°C), efforts were made to freeze buffalo semen. However, there was no effective extender formulation suitable for buffalo. It was not until 1972 that Pavinthran et al. reported a satisfactory freezability and non-success in freezing viable buffalo semen.

Thereafter, several laboratories experimented with different extender formulations, levels of cryoprotective agents, methods of glycerization, cooling and equilibration times, freezing and thawing rates and finally, fertility testing of the frozen semen of buffaloes.

Of the various extenders so far tried, Tris-yolk-glycerol is the most suitable extender in terms of post-thaw motility and fertility. The optimum level of glycerol in the extender media appears to be 6.5 - 7.0% when used as the sole cryoprotectant, although lower levels of glycerol can be used if other cryoprotective agents such as lactose or raffinose are present. The LN₂ vapor freezing method of extended semen in straws has become popular, while the production of frozen buffalo semen in pellet form is used in some buffalo AI stations. Pregnancy rates following the use of pelleted buffalo semen are comparable with those obtained using frozen-thawed semen in straws.

To date, the use of AI in water buffalo is part of the overall genetic improvement program. The only limitation to the wide-scale use of this technique, particularly in relatively dispersed buffalo populations in most of the small farms in Asia, is the development of a sustainable and effective system of AI service delivery.

Many of the techniques needed for the attainment of quality semen and higher pregnancy rate following AI in water buffaloes have been thoroughly described in published reports. The reported low efficiency of AI in water buffaloes is mainly the result of human factors such as inability to properly detect estrus, improper semen handling and usage in the field by technicians, and most common of all, poor management and nutrition of inseminated animals.

Control of Ovarian Function

Together with the development of AI techniques and freezing of buffalo semen, improved breeding management has been achieved through the control of ovarian function.

The discovery of the luteolytic effect of prostaglandin F₂ (PGF₂) has made synchronization a very useful tool in breeding management, particularly among buffaloes noted to express silent heat.

Various authors have reported the use of PGF₂ or one of its potent synthetic analogues in estrus control in buffaloes, often using an 11-day interval between two consecutives doses (Rao and Venkateswara Rao 1979; Bruce et al. 1988; Diaz et al. 1991), or in a single dose (Cruz et al. 1989), or in combination with GnRH for pre-determined AI (Aquino et al. 1989). Singh and Madan (1991) on the other hand, discussed the use of prostaglandin for estrus synchronization and the treatment of anoestrus and subestrus in the buffalo.

With regard to the prostaglandin dose required, it is evident from studies such as those of Rao and Venkatramaiah (1989) that the intravulvosubmucosal route can allow the effective dose of cloprostenol to be reduced to 20% (i.e. 100 ug) of the recommended intramuscular dose in subestrous Murrah buffaloes. By using this route, there is an immediate transfer of prostaglandin from the injection site to the ovarian artery resulting in luteolysis. The luteolytic effects of a small dose of cloprostenol (100 ug) administered by the intravulval
route in river buffaloes was also reported by Rao and Rao (1990). They found this dose to be as effective as the larger one (500 ug) given by intramuscular injection. The decline in progesterone concentration and the onset of estrus after prostaglandin treatment was found to be slower in buffaloes treated by the intravulvosubmucosal route (8 mg dose) than in those injected intramuscularly (25 mg dose) (Dhaliwal and Sharma 1990).

In estrus control, and more particularly, in the treatment of anestrus in buffaloes, intravaginal devices impregnated with progesterone (PRIDs and CIDRs) and ear implants impregnated with a potent progestagen (norgestomet) have been widely used (Saini et al. 1986; Singh et al. 1988; Subramaniam and Devarajan 1991; Luthra et al. 1994). In dealing with anestrus animals, gonadotrophin (usually PMSG) has often been administered at the time of progesterone/progestagen withdrawal.

For estrus synchronization and estrus detection in swamp buffaloes, Hill et al. (1992) observed that although CIDRs can be used in buffaloes, there can be a high rate of loss.

**Embryo Technologies**

Embryo transfer technology aims at harnessing the genetic potential of females. It is based on the ability to produce more embryos from superior donors than would be possible in natural breeding. The general concept is to accelerate the multiplication of superior animals through production, collection of embryos and transfer, utilizing ordinary recipient animals. Therefore, it follows that the efficiency of embryo production is a major limiting factor for the overall success of the embryo transfer program.

Buffalo embryos are currently being produced by both *in vivo* and *in vitro* techniques. In both systems, there is still a need to improve efficiency to reduce the cost of producing a calf. The current efficiencies are described below and the known factors affecting these are discussed.

**Follicular Population in Water Buffaloes**

As with other livestock species, the ovarian follicular population in water buffalo has been a subject of interest for some years. This is because the ovarian follicular pool determines how many follicles are available as potential sources of oocytes. In swamp buffaloes, Smith (1990) reported the number of ovarian follicles of various sizes to include also those atretic at different age groups (Table 1). Ovaries obtained from two-year old swamp buffaloes indicated a relatively high rate of transformation from the primordial follicle to growing follicles and up to tertiary follicles. In 7-8 year-old buffaloes, this massive transformation was not noticeable, and the average primordial follicular population was as high as 5,997. Among riverine buffalo heifers, the reported number of primordial follicles per ovary ranges from about 6,000 (Danell 1987) to 19,000 (Samad and Nasseri 1979).

The number of secondary follicles in the pubertal buffaloes indicates that transition of the growing follicles to secondary follicular stage was at a slower rate. The number of secondary follicles was only 7.56% of the growing follicles. The average numbers of secondary follicles in the adult and old animals were 14 and 8, respectively, and were 77.7% and 47.0%, respectively, of the number of growing follicles counted.

A decline in the number of tertiary follicles was also observed with age. The number of the tertiary follicles present in pubertal buffaloes was 67.2. This was significantly greater than those in adult and old buffaloes, at 9.0 and 6.67, respectively.

The transformation of primordial follicles to the growing stage and finally to the tertiary stage appears to be very inefficient. This inefficiency can also be seen in the relatively high level of atretic follicles measured as a percentage of the total follicles > 1.0 mm diameter. This pattern was also observed among riverine buffaloes (Danell 1987), and is higher than the levels of atretic follicles reported in cattle (Settergren 1964).

The combined effects of the small number of follicles and higher frequency of follicular atresia in buffaloes may mean that the number of available normal follicles can only be a fraction of what has been reported in cattle. This difference in the number of available ovarian follicles may explain in part the reported lower ovulatory response in buffaloes compared to that of cattle.

**In vivo Production of Embryos**

**Single Ovulation and Embryo Transfer (SOET)**

Attempts were made to produce embryos *in vivo* without superovulatory treatments. The objective was to harness the natural cycle to produce embryos following natural or artificial insemination. In essence, only a single ovulation, and therefore only one embryo, can be expected per flushing. This procedure does not entail exogenous hormones. Therefore, no disturbance in the physiological milieu of the donor animals is expected.
Singla and Madan (1990) collected embryos through SOET with 60% efficiency from 20 riverine buffalo donors. The efficiency of the flushing session in this technique, however, was limited by the estrous cycle of the animals, but high-quality embryos were harvested. Depending on the efficiency of collection and success of transfers, a donor can potentially produce 3-6 calves per year.

Multiple Ovulation in Buffaloes

Much attention has been focused on developing the most appropriate treatment for induction of multiple ovulation in water buffaloes. Trials have included tests on the kind of ovulatory hormone, hormone dosage, time of hormone administration and mode or pattern of administration. Results from such trials were not very consistent, while ovulatory responses were found to be lower than those achieved in cattle.

One major determinant of responsiveness to superovulatory hormones would be the number of healthy follicles available in the follicular pool. In cattle, Monnaux et al. (1983), defined these follicles as those with a diameter of >1.7 mm. Based on the data presented elsewhere in this paper, buffaloes are expected to have a smaller number of healthy follicles of the size considered to be responsive to hormonal stimulation. It is interesting to note that mares also exhibited limited response to superovulation (Woods et al. 1982). Horses are a species with a reported follicular population similar to that of buffaloes (Driancourt and Hariano et al. 1982).

Effect of Type and Dose of Hormone

The use of FSH and PMSG for superovulatory treatments have been the subject of early investigations (Drost et al. 1983; Karaivanov 1986; Alexiev et al. 1988; Sharifuddin 1988; Ventura 1991; Misra et al. 1994; Alonso et al. 1994). The point of reference has always been the number of ovulations, total number of embryos recovered and total viable embryos recovered following treatment with 2000 to 3000 IU PMSG or a total of 40 to 50 mg FSH. To date, it is clear that FSH is a better superovulatory hormone than PMSG. PMSG treatment has always been associated with a high incidence of unovulated follicles (Schallenberger et al. 1990; Cruz et al. 1992) and thus with a lower embryo yield (Table 2). Earlier reports also indicated that responses to FSH were 91.2%, compared to only 80.8% from PMSG treatments, in a large-scale trial (n=262) (Misra et al. 1994).

Improvement in embryo recovery and embryo quality following PMSG treatment may be achieved with the use of PMSG antiserum, as shown in cattle (Saumande and Chupin 1981). On the other hand, the development of a new purified FSH preparation has further improved the consistency of ovulatory response in buffaloes (Misra et al. 1994; Madan et al. 1996). This comes in the form of 600 mg NIH-FSH-P1 administered in 10 divided and decreasing doses at an interval of 12 hours. This is followed by PGF2 administered 72 hours after initiation of superovulatory treatment. This superovulatory regimen resulted in an average of 4.2 total embryos and 2.1 viable embryos per flushing (Misra et al. 1994).

Effect of Time of Hormone Administration

Superovulatory hormones are normally administered during the midluteal phase. Treatments are usually begun on Day 9-12. The concept of modifying the time of gonadotropin treatment relative to the stage of the luteal phase is based on the observation that follicular development in buffalo
(Danell 1987; Smith 1990) as well as in cattle, occurs in waves. This means that there are several periods throughout the cycle when follicles start to grow. Those that start to grow during the initial and middle of the luteal phase would normally get insufficient gonadotropin stimulation as a result of the negative feedback of the progesterone on the circulating FSH. In effect, the follicles recruited from the pool at these periods would get atretic before they reach ovulatory size.

Logically, therefore, follicles destined to grow towards the later part of the luteal phase would likely have an optimum environment. One advantage is that they are exposed to progesterone for a sufficient period prior-to-ovulation, compared to those follicles that grow during the early luteal phase. Evidence indicates that follicular cells need sufficient exposure to progesterone. This concept may also be expanded to cover the requirements for the normalcy of the oocytes. Such a necessity has been documented in the case of normal functioning of corpora lutea after induced ovulation (Cruz 1990).

Ovarian follicular development in water buffaloes treated on Day 9 or Day 15 of the cycle is shown in Table 3. While there was no dramatic difference between the two groups in the total number of surface follicles >1.0 mm, there was a significant number of follicles >5.0 mm on those that received PMSG on Day 9 of the cycle.

**Effect of Hormone Pre-Treatment**

The concept regarding the need to mobilize the small follicles to the stage considered to be responsive to superovulatory treatment has been the basis for trials on hormone pre-treatment prior to main superovulatory regimen.

The time for this pre-treatment, to be able to achieve its objective, has to be long enough to cover the required time for transit of follicles from antrum formation to formation of large size follicles. In cattle, based on mitotic index of granulosa cells, Scaramuzzi et al. (1980) estimated that about 22 days is required for a bovine follicle to grow from small antral (0.4 mm diameter) to large antral size (10.0 mm diameter). This would suggest that pre-treatment may be suitable at least two days prior to the main hormone treatment.

Test on the administration of 12 mg FSH given in divided doses of 3 mg every 12 hours prior to the main FSH treatment resulted in a significant improvement in ovulation response, an increase equivalent to 30% above the control group (Table 4).

**Embryo Harvest From Superovulated Riverine Buffaloes**

Commercial scale projects involving superovulation and embryo collection in riverine buffalo have been carried out in India (Misra et al. 1994). The efficiency of the treatment regimen in terms of average embryo harvest per treatment was 2.61, with 1.43 viable embryos. Over a period of five years, the system improved from a total embryo yield of only 1.77 to 3.83, and the average number of viable embryos from 0.92 to 2.13 (Table 5).

**Effect of Repeated Superovulatory Treatments**

The possible negative effects of repeated superovulatory treatments on the hormonal and physiological milieu of donor animals have received attention. One of the clear examples on demonstrating the effect of this series of FSH treatments is shown in Table 6.

Of the 41 buffaloes that were repeatedly treated for superovulation at an interval of 77 days on the average, there was a marked progressive decline in

---

**Table 2. Ovulatory response of water buffaloes to FSH or PMSG**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>n</th>
<th>Total CL embryos</th>
<th>Total viable embryos</th>
<th>Total embryos per flush</th>
<th>Total viable embryos per flush</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (600 mg MIH-FSH-Pf)</td>
<td>97</td>
<td>680</td>
<td>398</td>
<td>203</td>
<td>4.1</td>
</tr>
<tr>
<td>PMSG (3000U)</td>
<td>25</td>
<td>94</td>
<td>40</td>
<td>14</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Misra et al. 1994
was reported to be higher during summer than in other periods of the year (Cruz 1992).

On several occasions, the low levels of circulating estrogen during treatment have been associated with low embryo yield (Madan et al. 1996; Misra 1993; Rao 1994). To some extent, this low estrogen level among poorly responding animals is just a reflection of poor follicular development.

Recent results suggested that buffaloes respond better to conventional superovulation treatment when treated in the absence of a dominant follicle, or when the dominant follicle is in a regressing or plateau phase (Taneja et al. 1995). It appears that the presence of a dominant follicle might be deleterious to the process of recruitment and passage of follicles from smaller to large size during superovulation, a case earlier reported in cattle (Guibault et al. 1991).

**Other Factors Affecting Ovulatory Response**

The seasons seem to affect the response to superovulatory treatment, with summer having a depressive effect (Misra 1995; Rao et al. 1994; Matharoo and Singh 1994). High temperatures in summer may have some influence on the hormonal milieu of buffaloes, thus affecting the responsiveness of the ovary to exogenous hormone stimulation. Summer conditions affect gonadotropin secretion, and would therefore theoretically affect ovarian function. In fact, the incidence of inactive ovaries was reported to be higher during summer than in other periods of the year (Cruz 1992).

On several occasions, the low levels of circulating estrogen during treatment have been associated with low embryo yield (Madan et al. 1996; Misra 1993; Rao 1994). To some extent, this low estrogen level among poorly responding animals is just a reflection of poor follicular development.

Recent results suggested that buffaloes respond better to conventional superovulation treatment when treated in the absence of a dominant follicle, or when the dominant follicle is in a regressing or plateau phase (Taneja et al. 1995). It appears that the presence of a dominant follicle might be deleterious to the process of recruitment and passage of follicles from smaller to large size during superovulation, a case earlier reported in cattle (Guibault et al. 1991).

**IN VITRO PRODUCTION OF BUFFALO EMBRYOS FROM OVARIES OF SLAUGHTERED ANIMALS**

The yield of immature oocytes that would qualify for *in vitro* maturation from the ovary of a

---

**Table 3.** Mean SD frequency of ovarian surface follicles of various sizes 2 days after buffaloes in estrus were treated with PMSG (3000 IU) during on Day 9, (n=19) or Day 15 (n=16)

<table>
<thead>
<tr>
<th>Follicle size (mm)</th>
<th>Time of PMSG treatment</th>
<th>Day 9</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td></td>
<td>1.224</td>
<td>0.000</td>
</tr>
<tr>
<td>1.0 - 2.5</td>
<td></td>
<td>6.622</td>
<td>5.332</td>
</tr>
<tr>
<td>2.6 - 5.0</td>
<td></td>
<td>3.827</td>
<td>6.673</td>
</tr>
<tr>
<td>5.1 - 7.5</td>
<td></td>
<td>1.417</td>
<td>3.320</td>
</tr>
<tr>
<td>7.6 - 10</td>
<td></td>
<td>3.234</td>
<td>0.304</td>
</tr>
<tr>
<td>&gt;10.0</td>
<td></td>
<td>3.227</td>
<td>0.304</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>19.417</td>
<td>16.026</td>
</tr>
</tbody>
</table>

**Table 4.** Mean SD number of ovulation and number of unovulated follicles >5mm in pFSH treated buffaloes with or without pFSH priming

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Ovulation</th>
<th>Unovulated follicles &gt; 5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without priming</td>
<td>16</td>
<td>2.0054a</td>
<td>2.8098a</td>
</tr>
<tr>
<td>With priming</td>
<td>8</td>
<td>2.60074b</td>
<td>2.8173a</td>
</tr>
</tbody>
</table>

a,b - Means in the same column with dissimilar superscripts differ (p<0.05)
slaughtered buffalo is rather limited in number. Depending on the efficiency of aspiration and the oocyte grading system, the oocyte harvest per ovary can range from 0.46 (Totey 1991) to 3.0 (Duran et al. 1996), an average of only 1.5 per ovary.

**In Vitro Oocyte Maturation**

The ability of immature buffalo oocytes aspirated from ovaries collected from slaughtered buffaloes to mature in vitro is influenced to a large degree by the maturation media and supplements. It appears that benefits from the supplements are media-dependent. A better maturation rate was seen from FSH and estradiol supplementation on TCM 199 media than on Hams F-10. Benefit from LH supplementation is more pronounced when it is added to Hams F-10 than to TCM 199 (Totey et al. 1992).

In our laboratory, the effect of LH supplementation on nuclear maturation of buffalo oocyte has been assessed. Increasing the level of LH supplementation from 0 to 10 g/mL in TCM 199 media supplemented with FSH and estradiol brought about an increasing nuclear maturation rate (Table 7).

Studies that resulted in higher mean maturation rate (81.7% - 86.0%) in buffalo oocytes were essentially TCM-199 supplemented with FCS and FSH with or without estradiol (Singh et al. 1989; Totey et al. 1992; Ocampo et al. 1996).

**In Vitro Fertilization**

Evaluation of culture media for IVF suggested that BO medium supported higher fertilization and cleavage rates than MTALP medium (Totey et al. 1992; Madan et al. 1994; Ocampo et al. 1996) with
fertilization and cleavage rates of 29.8% - 78.2% and 27.6% - 68.5%, respectively.

There was a high occurrence of polyspermy observed among IVF buffalo oocytes. Increasing sperm concentration from 1, 5 and 10 x 10^6 increased polyspermy from 24.0%, 43.2% and 64.0%, respectively (Ocampo et al. 1996).

The efficiency of embryo production through in vitro procedure is shown in Table 8. It is clear that efficiency has to be improved at the level of maturation, as it perhaps reduces incidence of polyspermy. One critical aspect of a buffalo oocyte IVM/IVF system is improving the culture system for the development and formation of viable and transferable blastocysts.

**IN VITRO PRODUCTION OF BUFFALO EMBRYOS ASPIRATED FROM LIVE ANIMALS**

Following successful trials of ultrasound guided oocyte aspiration from living cattle, commonly known as ovum pick-up (OPU), efforts were made to duplicate the same technique in buffalo (Boni et al. 1994). This technique allows repeated retrieval of oocytes from the best female animal without sacrificing the animal for future production. OPU technique can complement IVM/IVF to produce genetically superior embryos.

Oocyte Harvest

The average yield of immature cumulus cell oocyte complexes (COC) following in vivo aspiration on non-supervoluted buffaloes using an ultrasound guided needle, was 1.33 per ovary per collection (Boni et al. 1994). Of these oocytes, only 31.3% were classified as suitable for in vitro embryo production. Taking into account the number of follicles aspirated and the harvest of oocytes, the system was about 31.9% efficient.

Among animals treated with FSH, in vivo oocyte aspiration yielded an average of 3.0 oocytes per ovary per aspiration, with an efficiency of 44.4% (Boni et al. 1994). Laparotomy done in pre-pubertal swamp buffaloes (8-12 mo.) treated with PMSG or FSH yielded an average total embryo of 4.6 and 8.3, respectively (Techakumpu et al. 1995).

Oocyte Quality

Factors that may affect oocyte harvest from buffalo through ultrasound guided aspiration include the physiological status of the donor, the time of ovum pick-up relative to postpartum, the presence of a dominant follicle and the day of the cycle. The effect on oocyte recovery of the physiological status of donor and days postpartum at time of OPU is shown in Table 9.

Note that although the number of samples is limited, the physiological state of the ovary less affected seems to influence the quality of the oocyte more than the post partum days at time of OPU. Fewer post partum days yielded a greater number of cumulus cell oocyte complex (COC) and thus a higher rate of COC recovery at 76.2% vs 24.3%.

Also, the day of the cycle at the time of OPU appears to have influenced the quality of COC, in that OPU conducted on the 6th day resulted in an average of 46.8% good quality COC, while OPU on days 7-11 yielded only 25% good quality COC.

**OPU Plus IVM/IVF Oocytes of Buffalo**

The yield of immature oocytes from buffaloes through OPU in recent trials are shown in Table 10. Of the oocytes aspirated, the maturation rate in vitro ranged from 44.5% to 58.3% (Tavares et al. 1997; Boni et al. 1997).

**Efficiency of OPU + IVM/IVF**

Following the transferable embryo production

---

**Table 7. Nuclear maturation of buffalo oocytes in TCM 199 supplemented with FSH and Estradiol and varying LH concentrations**

<table>
<thead>
<tr>
<th>LH, g/mL</th>
<th>Cultured</th>
<th>No. of oocytes matured</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>243</td>
<td>171</td>
<td>(71.3)</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>107</td>
<td>(70.3)</td>
</tr>
<tr>
<td>10</td>
<td>690</td>
<td>570</td>
<td>(82.6)</td>
</tr>
</tbody>
</table>

---
efficiency presented in Table 11, (Zicarelli et al. 1997), it can be deduced that in a year's time, and with an OPU interval of 3-4 days, it is possible to expect an average yield of about 15.7 to 34.6 transferable embryos per buffalo cow. This efficiency is considered to be less than half that expected from cattle.

The use of OPU + IVM/IVF could notably increase embryo production, thereby enabling a greater female contribution to genetic improvement. It seems that remarkable genetic improvement can be obtained using these techniques, while the generation interval would decrease from 6.28 years to only 3.25 years.

Comparing estimates of genetic gain of an entire population, it can be seen that using a shared nucleus with OPU + IVF and a closed nucleus with AI is about 30% and 25% greater, respectively, than a selection using a progeny test for the entire population.

Table 8. Efficiency of buffalo oocyte IVM/IVF

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocytes</td>
<td>351</td>
<td>100</td>
</tr>
<tr>
<td>Nuclear maturation rate</td>
<td>289</td>
<td>82.3</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>203</td>
<td>57.6</td>
</tr>
<tr>
<td>Normal fertilization rate</td>
<td>121</td>
<td>34.4</td>
</tr>
<tr>
<td>Polyspermic fertilization rate</td>
<td>82</td>
<td>23.3</td>
</tr>
<tr>
<td>No. of oocytes cultured</td>
<td>183</td>
<td>100</td>
</tr>
<tr>
<td>Cleavage rate (2-4 cells)</td>
<td>98</td>
<td>53.5</td>
</tr>
<tr>
<td>Blastocyst formation rate</td>
<td>21</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Table 9. Recovery rate (ROR) cumulus cell-oocyte complex (COC) recovery by OPU from cyclic and acyclic water buffaloes at different days post-partum

| Cyclic   | 1.50 | 9.3 | 7.3 | 29.9 | 78.2 |
| Cyclic   | 500  | 5.0 | 1.0 | 25.0 | 20.0 |
| Acyclic  | 98   | 8.7 | 6.5 | 15.3 | 74.3 |
| Acyclic  | 745  | 5.3 | 1.5 | 25.0 | 28.6 |

Boni et al. 1995

PREGNANCY FOLLOWING EMBRYO TRANSFER IN WATER BUFFALO

Pregnancy rates following non-surgical transfer of in vivo-derived buffalo embryos in early trials were only 9.2% (Kurup et al. 1988), 17.9% (Drost et al. 1988) and 18.3% (Alexiev et al. 1988), except for the 100% success by Drost et al. in transferring a single embryo in 1983.

In recent years (1989-1993), transfers of 469 in vivo-derived buffalo embryos resulted in only 17.0% pregnancy (Misra et al. 1994). Out of these pregnancies, 46 calves were born. This is equivalent to 9.8% of the total embryos transferred, a level similar to those earlier obtained (Drost et al. 1988; Alexiev et al. 1988).

This low rate of birth was associated with a high rate of pregnancy failure of up to 40% (Misra et al. 1994).

Very recently, single transfers of 76 grade 1 and 2 in vivo-derived buffalo embryos resulted in 30.2% pregnancy (Campanile et al. 1995). The average pregnancy rate of the fresh or frozen-thawed
embryos (31.4% vs. 28.0%) or between morula or blastocyst (29.4% vs. 30.5%) was not very different. The interesting observation was that the pregnancy rate was significantly higher in recipients with a smaller CL (<1.0 cm) at the time of embryo transfer than those with a CL > 1.3 cm (Campanile et al. 1995).

The limited number of transfers of in vitro-derived buffalo embryos makes it difficult to assess the true pregnancy rate at the moment. In one study, a 30% (3/10) pregnancy rate was reported (Totey et al. 1996). Taking cues from the data on cattle, the successful pregnancy rate of IVM/IVF embryos is lower than in vivo-derived embryos. It has been clearly demonstrated that the reduced inner cell mass in blastocysts derived from IVF are indicative of reduced viability (Iwasaki et al. 1990; Willadsen and Polge 1981). There are some indications in cattle that the pregnancy rate from IVM/IVF embryos obtained from pre-pubertal animals was lower than if the donors had reached puberty (Damiani et al. 1996).

**CONCLUSION**

Water buffalo will remain an important component of the Asian economy. Genetic improvement in this important animal resource is focused on its role as a major provider of milk and source of meat in this part of the world. The limitation to genetic improvement imposed by inherent biological parameters such as the long generation interval can be overcome with the use of recently developed reproductive biotechniques.

Artificial insemination will remain a major technology for use in buffalo genetic improvement in the future. The need to improve AI efficiency is mainly related to improving the human-related factors such as appropriate timing of AI, suitable semen handling and hygiene.

The low population of ovarian follicles sets a limit to the efficiency of superovulatory regimens

---

**Table 10. Efficiency of cumulus-oocyte complex recovery by OPU and its maturation in vitro**

<table>
<thead>
<tr>
<th>No. of buffalo heifers</th>
<th>5</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspiration sessions</td>
<td>15</td>
<td>92</td>
</tr>
<tr>
<td>No. of aspirated follicles</td>
<td>227</td>
<td>-</td>
</tr>
<tr>
<td>Ave. follicle/animal/session</td>
<td>3.03</td>
<td>7.70</td>
</tr>
<tr>
<td>Recovery rate, %</td>
<td>1.35</td>
<td>4.49</td>
</tr>
<tr>
<td>Maturation of COC in vitro</td>
<td>44.5</td>
<td>58.3</td>
</tr>
</tbody>
</table>

Tarares et al. 1997
Boni et al. 1997

**Table 11. Efficiency of production of embryos through in-vivo (ET), IVM/IVF from slaughtered animals (IVM/IVF) and OPU/IVM/IVF (OPU/IVM/IVF) in cattle and buffalo**

<table>
<thead>
<tr>
<th></th>
<th>ET</th>
<th>IVM-IVF</th>
<th>OPU-IVM-IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Buffalo</td>
<td>Cattle</td>
</tr>
<tr>
<td>% Efficiency at S.O.</td>
<td>88</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Embryos per flushing or per OPU session</td>
<td>7.4</td>
<td>1.8</td>
<td>1.5 - 5</td>
</tr>
<tr>
<td>T.E./flushing or session</td>
<td>4.4</td>
<td>1.7</td>
<td>0.8 - 3</td>
</tr>
<tr>
<td>Repetition interval (days)</td>
<td>75</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Embryos obtainable in 75 or 100 days</td>
<td>14.8</td>
<td>3.6</td>
<td>1.5 - 5</td>
</tr>
<tr>
<td>T.E. obtainable in 75 or 100 days</td>
<td>8.8</td>
<td>3.4</td>
<td>0.8 - 3</td>
</tr>
</tbody>
</table>

Zicarelli (1997)
for buffaloes. There is a need, therefore, to develop a more efficient system in order to harness the available potential oocytes and maximize the utility of the superior members of the population.

As an alternative, embryos can be produced in vitro through the aspiration of oocytes in vivo from superior donors and fertilizing the same in vitro. Oocyte fertilization and culture in vitro appear to be the weak points in this system. Efficiency has to be improved considerably if this technology is to become a practical tool in buffalo improvement. There are also various ways to maximize the efficiency of use of already available embryos, such as splitting the embryos prior to transfer.

**REFERENCES**


Production of PMSG antiserum in cattle; Assay of inhibitory activity and use in superovulated heifers. *Theriogenology* 15: 108 (Abst.).


