A Brief Review of Assisted Reproduction and Artificial Insemination in Non-domestic Avian Species

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Assisted reproductive technologies and artificial insemination are routinely used with great success in many mammalian species (Durrant, 2009). However, when you compare the level of research and collective information available it becomes clear that assisted reproduction in avian species is still in its infancy (Blanco et al., 2009). Significant advancements have been made in the artificial breeding techniques in domesticated species (Zhang, 2006), however research in non-domestic species is, by comparison, very limited (Samour, 2002). Approximately one third of the worldwide psittacine population are endangered (DellaVolpe et al., 2011) with at least 11% of all bird species being threatened with extinction (Saint Jaime, 2000). Captive breeding programs face challenges of animal compatibility, distance to transport live animals and low genetic diversity within the captive population (Durrant, 2009). The potential to greatly improve the success of these conservation programs as well as the possibility of developing a reproductive veterinary service for private aviculturists warrants further research into assisted reproduction. Recent literature on the use of GnRH analogues, semen collection, artificial insemination techniques, timing of insemination and semen storage are reviewed herein.

THE USE OF HORMONES

Avian reproductive behaviour is primarily induced by changes in day length that stimulate Gonadotropin Releasing Hormone (GnRH) release (Aiudi et al., 2009). This leads to the subsequent release of luteinizing hormone and follicle stimulating hormone; ultimately driving reproductive behaviour (Bedecarrats et al., 2006). There are two distinct forms of GnRH in birds with a third also being reported (Bedecarrats et al., 2006). These are all thought to play an important role in the aforementioned hormone cascade (Bedecarrats et al., 2006).

Neuroendocrine conditioning of reproduction in avian species could provide a means of increasing reproductive success among captive animals (Costanini et al., 2009; Lovas et al., 2010). The use of a slow release GnRH analogue (buserelin acetate) into budgerigars yielded significant increases in rates of egg-laying, fertile eggs produced and number of chicks hatched successfully (Costanini et al., 2009) (Figure 1).

The hormone implant resulted in higher levels of steroid metabolites (testosterone in males, oestrogen in females) passed in the faeces; however it appeared to still allow for normal follicular development (Costanini et al., 2009). This is important as testosterone is the primary sex steroid in the avian testes and is thought to play a crucial role in reproductive behaviours (Lovas et al., 2010). Buserelin (trade name Receptal®, Intervet, Australia) has also been shown to induce a significant

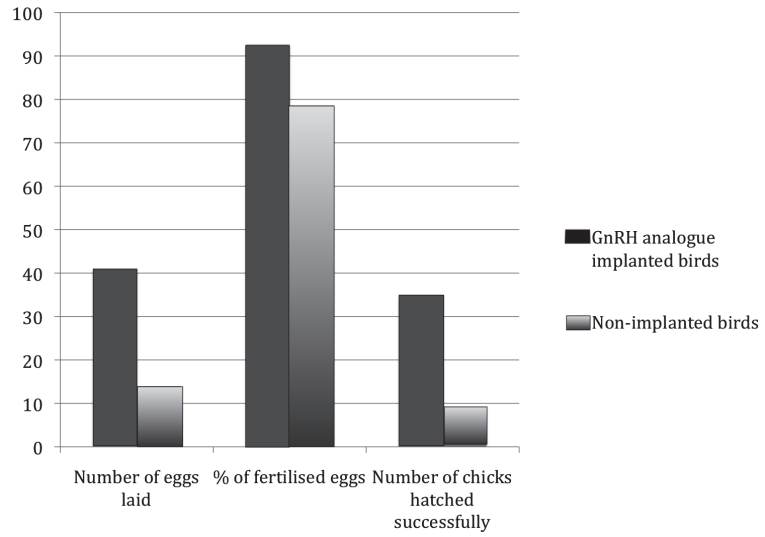

Figure 1 – The effect of buserelin on Budgerigar reproduction (adapted from Costanini, et al., 2009)

increase in testosterone concentration in male cockatiels and sulphur crested cockatoos (Lovas et al., 2010), and to increase annual egg production and clutch number in ostriches (Aiudi et al., 2009). The promising results seen with the use of the GnRH analogue may provide a less-invasive alternative or adjunct to artificial insemination however further research is needed.

SEMEN COLLECTION

Semen availability, collection and quality have been shown to be the most limiting factors in achieving successful artificial insemination (Zhang et al., 2006; Blanco et al., 2009). A number of semen collection techniques have been attempted, including voluntary copulation onto special devices by human imprinted birds and electro-ejaculation (Samour, 2004; Immler and Birkhead, 2005; Blanco et al., 2009). Another technique, that has been adapted from semen collection in domestic chickens (Samour, 2004), involves the use of an abdominal and/or cloacal massage (Samour, 2002; Zhang, 2006; Blanco et al., 2009). This generally involves restraining the male bird either by an operator (Figure 2) or in a specially made collection station (Figure 3). The advantages of the collecting station are that it is thought to allow for a more natural copulation position and slightly more freedom for the birds to move, potentially reducing the stress placed on the animal and increasing the likelihood of obtaining a viable sample (DellaVolpe et al., 2011).

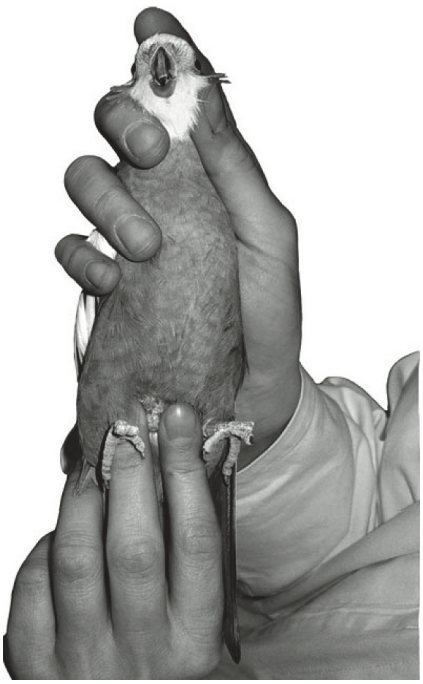

Figure 2: Manual operator restraint (Stelzer et al., 2009)

The two main massage techniques that have achieved successful semen collection are the cloacal massage and abdominal massage technique (Blanco et al., 2009; DellaVolpe et al., 2011). In both of these techniques the cloaca is generally cleaned and flushed with warm saline or water prior to commencing the

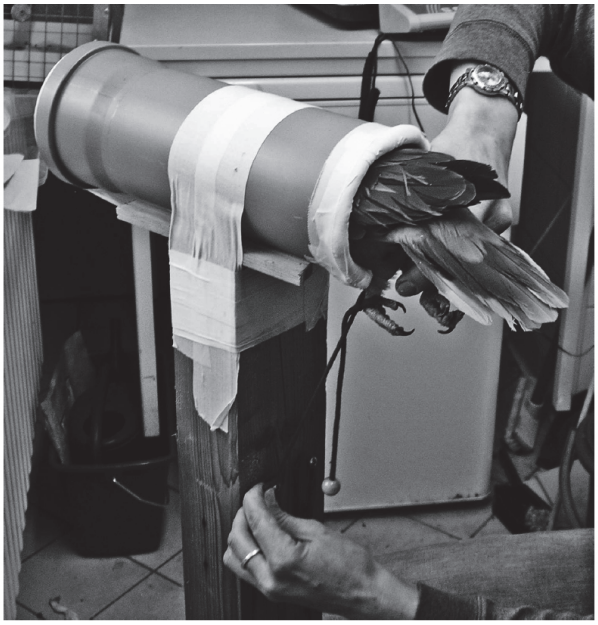

Figure 3: Collecting Station (Dellavolpe et al., 2011)

massage to reduce faecal and urate contamination (DellaVolpe et al., 2011). The cloacal massage technique involves placing the operator's fore and middle finger on either side of the cloaca with the thumb positioned dorsally near the uropygial gland (Stelzer et al., 2009). From this position gentle rhythmic pressure is applied to the cloaca until ejaculation is achieved (Stelzer et al., 2009). The abdominal massage technique involves gentle but rapid stroking of the base of the tail, abdomen and lower back (DellaVolpe et al., 2011). This is continued until a cloacal reaction occurs. At this point the handler gently applies pressure to both sides of the cloaca to express any semen that is present (Samour, 2002; Blanco et al., 2009). In both techniques the semen is generally collected using a dry, non-heparinised microhaematocrit capillary tube (Blanco et al., 2009).

The most significant problem with the massage techniques is a high contamination rate (DellaVolpe et al., 2011). However it must be noted that micro-organisms are generally present in every ejaculate making the aim of obtaining sterile semen unrealistic (Thibier and Guerin, 2000). In addition to the microorganisms, one study has shown that 52.6% of samples obtained were contaminated with urates, urine, blood or faecal material; all of which have been shown to decrease sperm viability (DellaVolpe et al., 2011). The presence of contamination also increases the risk of introducing pathogenic bacteria, most commonly *Escherichia. coli*, into the female reproductive tract if artificial insemination is attempted (Blanco et al., 2009).

Methods to decrease contamination include withholding food and water for 6-8 hours before collection and ensuring that the bird moves around the cage before it is captured to induce defecation (DellaVolpe et al., 2011). When these techniques were implemented the contamination rate was much lower (DellaVolpe et al., 2011). A successful technique has also been developed in birds of prey to reduce urine contamination that involves washing the semen obtained (Blanco et al., 2002). Equal volumes of semen and diluent are mixed together and centrifuged; the supernatant is then decanted and the procedure is repeated (Blanco et al., 2002). Using this technique the level of motile spermatozoa increased and successful artificial insemination was achieved (Blanco et al., 2002).

Other variables that must be taken into consideration are the timing of collection, how often to collect and seasonal variation in sperm production (Blanco et al., 2009). It has been demonstrated that collection attempts in the early morning, shortly after the bird has woken up, appear to improve semen yields (DeMatteo et al., 2004). Lovas et al. (2010) demonstrated that individual birds of the same species can have significantly varying testosterone concentrations at any given time even if housed in the same conditions. It also appears that semen production may only occur at certain times of the year with potential intermittent non-productive periods (Samour, 2002; Zhang, 2006). Little is known about the optimal frequency at which semen should be collected and it is likely that this will vary greatly from species to species. One study used a twice weekly routine with varying success

(DellaVolpe et al., 2011).

The seasonal variation in sperm production will also differ greatly between species and is an important factor that cannot be overlooked. A good understanding of the natural mating behaviours and breeding season of the individual species is needed to increase the chances of success (Saint Jaime, 2002).

FEMALE INSEMINATION TECHNIQUES

The avian female reproductive tract is inherently complex (Figure 4) and a sound knowledge of the individual species anatomy is vital to success (Blanco et al., 2009). Once this has been accomplished the first step towards artificial insemination is to develop a means of adequate restraint of the female bird. The availability and practicality of using Isoflurane to induce general anaesthesia means that it is often used for a range of reproductive related procedures if prolonged restraint is necessary (Blanco et al., 2002; Lovas et al., 2010).

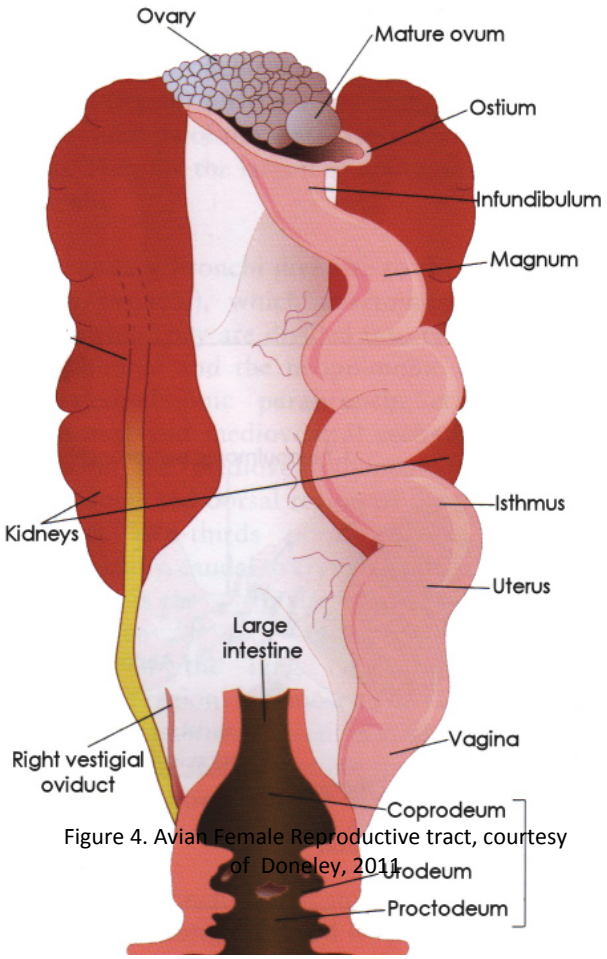

Figure 4. Avian Female Reproductive tract, courtesy of Doneley, 2011

Once appropriately restrained the next step is to determine the optimal insemination site, for this there are generally three main possibilities: cloacal, intravaginal or intramagnal (Blanco et al., 2009).

The advantages of cloacal insemination are that it is quick, easy and potentially less stressful if done while the bird is conscious (Blanco et al., 2009). The main disadvantage to this technique is that the semen can be easily expelled resulting in decreased chances of fertilisation (Blanco et al., 2009).

The intravaginal technique involves visualising the opening of the oviduct into the cloaca (Blanco et al., 2009). This can be achieved by stabilising the cloaca and firmly retracting the pericloacal skin while at the same time gently applying pressure to the lower abdomen to allow cloacal eversion (Blanco et al., 2009). An alternative approach is to use a suitable sized vaginal speculum or endoscope to open the cloaca and allow visualisation (Blanco et al., 2009). In immature birds the opening of the oviduct is covered by a membrane (Doneley, 2011). It is important to remember that in many birds the opening to the functional oviduct will be on the left side of the cloaca due to their reproductive anatomy. Once visualised the semen is deposited just inside the opening into the vagina (Blanco et al., 2009).

The third approach to be considered is the more invasive intramagnal route. This has the benefit of depositing the semen in the area adjacent to the infundibulum, which is thought to be where

fertilisation takes place (Doneley, 2011). Once the oviductal opening has been visualised an appropriately sized catheter is inserted into the vagina using a flexible 2mm endoscope to allow visualisation of the uterine lumen (Blanco et al., 2002). It is thought that the oviductal opening is easier to visualise after the first egg has been laid (Samour, 2002). The catheter is then passed up to a predetermined length based on previous post-mortem findings from similar species or as a result of contrast studies (Blanco et al., 2009). In budgerigars it has been found that the catheter needs to be inserted to an approximate depth of 20mm (Samour, 2002). The semen is then deposited by attaching a syringe containing the semen to the catheter and using an air embolus to push the semen out the end of the catheter (Blanco et al., 2009). Disadvantages to this technique are that it is more invasive, has an increased risk of introducing infection or causing damage, and it allows 'unfit' sperm that would normally be expelled by the vagina to reach the higher areas of the oviduct ultimately increasing the risk of embryonic mortality (Blanco et al., 2009).

Regardless of the insertion point chosen it is paramount that sterile equipment is used. As previously stated the possibility of introducing infection from the semen sample must be considered given the high likelihood of faecal contamination (Thibier and Guerin, 2000). The addition of antibiotics to the semen extender (Donoghue, et al. 2004) or using washed semen (Blanco et al., 2002) may be warranted to help decrease this risk.

TIMING OF INSEMINATION AND STORAGE OF SEMEN

The time of year that the insemination is performed is important and is best undertaken in the early peak of the breeding season once the animal has been adequately cycled with appropriate environmental and social stimulation (Blanco et al., 2009). Vasectomised males were needed to provide adequate stimulation to allow normal reproductive behaviour (lay eggs, carry out incubation and rear chicks) in female budgerigars (Samour, 2002). While the time of year is important the exact time in relation to ovulation may not be as crucial as first thought. The reason for this is that many, if not all, avian species have specialised tubular invaginations of the surface epithelium at the anterior end of the vagina known as sperm storage tubules (Birkhead and Moller, 1993; Blanco et al., 2002; Blanco et al., 2009). The exact period of viable sperm storage is unknown for most species however fertile eggs have been produced 6 days after artificial insemination in whooping cranes (Jones and Nicolich, 2001) and up to as long as 45 days after artificial insemination in the domestic turkey (Blanco et al., 2009). Further research is needed to determine individual species average sperm storage capacity (Zhang, 2006).

In an ideal situation the use of fresh undiluted high concentrate semen is generally considered optimal (Blanco et al., 2002). However this is not always practical and methods to allow semen preservation need to be considered. Semen cryopreservation of avian spermatozoa has been achieved and there are many variables to consider (Sontakke et al., 2004). These include the choice of cryoprotectant, cooling rate, freezing temperature and thawing conditions (Sontakke et al., 2004). Successful artificial insemination leading to the production of viable offspring using frozen-thawed semen has been achieved in range of species including the golden eagle (Blanco et al., 2009), budgerigar (Samour, 2002) and in a number of pheasant species (Saint Jaime et al., 2002). However optimal conditions needed to achieve this appear to differ between species, and further research is needed to determine whether it would be successful in all species.

CONCLUSION

The use of assisted reproductive technologies and artificial insemination in avian species has to be more refined before it could be easily integrated into conservation programs or private practice. However initial results are promising with successful artificial insemination being achieved in a range of avian species and in a few select avian conservation programs; notably a wild Kakapo population (Robertson, 2009). Artificial insemination may provide a means of increasing reproductive success and in some cases it has been shown to produce higher fertility levels than some naturally copulating populations (Gee et al., 2004). There is also the exciting potential for sperm 'cryobanks' to be developed for a range of threatened avian species (Saint Jaime et al., 2002) potentially providing a 'safety net' against species extinction.

The use of captive breeding and assisted reproductive technologies may be the only hope for the survival of many avian species (Samour, 2002). The benefits of increasing the success of species preservation programs and increasing genetic diversity within these programs are great and warrant further pursuit.

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