Semen collection for animal reproduction

Semen collection is the extraction of semen or spermatozoa from the penis to be used for artificial insemination and in vitro fertilisation. In koalas, electro-ejaculation is the more preferred method to using an artificial vagina to stimulate ejaculation (Rodger et al., 2009). However, electro-ejaculation produces semen that varies in its biochemical composition, such as pH and the concentration of the spermatozoa (Johnston et al., 1997) as well as being an invasive technique that involves the a 4. 5 – 5mm diameter rectal probe or an endotracheal tube administrating a small electrical stimuli and requires a general gaseous aesthesia to reduce stress to the koala (Allen et al., 2008). Electro-ejaculation is well established in most herbivore marsupials, but only artificial vaginas have successfully collected semen from koalas (Rodger et al., 2009). To use an artificial vagina, a sexually mature male must be surround by a group of sexually mature females to stimulate arousal, with a female that is displaying oestrus behaviour placed above the male on a vertical pole to stimulate thrusting behaviours (Johnston et al., 1997). The downside to using an artificial vagina is that the male needs to display and sexual interest in the female, and a lack of libido is one complication that replaces thrusting behaviour with an aggressive one (Johnston et al., 1997). Other complications include the contamination of urine in the semen, reduced thrusting of the penis when the artificial vagina is placed over it, no semen produced despite the interest from the male, partial ejaculations and non-compliant females (Johnston et al., 1997). Some times more than one attempt is needed when using the artificial semen to get a complete ejaculation (Johnston et al., 1997). All these complications show that electro-ejaculation, despite being invasive, gives us the better chance of collecting semen.

In elephants, a more manual approach is taken when collecting semen, however similar to the koala; chemical resistants are used to lessen the stress (Portas et al., 2007). The sedatives used include xylazine, butorphanol, yohimbine and naltrexone are applied intravenously (Portas et al., 2007) while the koala inhales a gaseous aesthetic. Unlike the koala, physical restraints in the form of straps are applied over the hind limbs of the elephant proximal to a secured tarsus on the walls of the chute as well as behind the bars of the shoot, secured to steel post at the front of the shoot to prevent movement from the elephant (Portas et al., 2007). To manually stimulate ejaculation, a rectal massage of the accessory glands, particularly the prostate, and pelvic urethra for around 10 minutes allows for the erect penis to prolapse from the sheath, in which a plastic collection sleeve with a 50mL collection funnel is placed at the end, allowing for the collection of semen (Portas et al. 2007, Saragusty et al. 2009). Like the koala, this method is invasive, as you have to enter from the rectum to get to the accessory glands. This method has become well-established and most common method of semen collection in elephants. Electro-ejaculation has also proved to be effective technique for semen collection in elephants, replacing the manual stimulation of the accessory glands, which is the same technique used for koalas, though an ultrasound is needed to locate the glands in elephants and to avid the bladder to avoid urine contamination (Hermes et al., 2013). To achieve ejaculation, a voltage of 15 – 30V and amperage of 450 – 1000mA with 12 – 16 electrical stimuli is needed (Hermes et al., 2013).

Evaluation of the semen evaluation assesses the quality of the semen in terms of the percentage of motility, spermatozoa concentration and the volume of the ejaculation. In koalas, a phase contrast microscope at 400X with a warm stage set at the temperature of a koala (35 ^{o}C) is used to assess the percentage of progressive motile spermatozoa (Allen et al. 2008, Johnston et al. 2012). Dual florescent staining technique determines the plasma membrane integrity and quantifies the incidence of sperm chromatin relaxation, when sperm that has been thawed show a tendency to swell up using two vital stains (Johnston et al., 2012). SYBR-14 that permeates the plasma membrane that is not damaged and stains the nuclei florescent green while propidium iodide permeates a damaged plasma membrane and causes florescent red staining, both observed under a fluorescein isothiocyanate 465-495 excitation filter, DM-505 dichroic mirror and BA 515-555 barrier filter (Johnston et al., 2012). A Makler sperm counter chamber is used to estimate the concentration of the fresh semen sample (Allen et al., 2008).

In elephants, volume in noted and sperm concentration is estimated using a Neubauer hemocytometer while a dark field microscope using 10X objectivity with a heating stage set at 37 ^{o}C to evaluate sperm motility (Hermes et al., 2013). Slides were prepared with a total of 100 strained spermatozoa per slide, using Kovács-Foote staining technique to assess for acrosome integrity, with each slide being categorized as intact or reacted, sperm morphology being observed and viability, each slide being categorized as “ dead” or “ viable” (Hermes et al., 2013). However, new technology, such as using a spectrometer calibrated to measure the

spermatozoa concentration as well as a vapour pressure osmometer determining osmolality (Kiso et al., 2010) are becoming more prevalent as technology keeps on advancing. Both koalas and elephants evaluate spermatozoa for motility, concentration and viability, however in elephants, spermatozoa are also assessed for acrosome integrity, morphology and osmolality while koalas look at plasma membrane integrity.

Freezing or chilling the semen allows for using the semen later and even exporting the semen to other parts of the country as well as internationally. In koalas, extenders, used for cryopreservation, where semen is placed in straw-like tubes that are frozen in liquid nitrogen vapours or in a programmable freezer (Allen et al., 2008). The extender for a koala, maintain pH control through a tris-citrate buffer, have a glycerol concentration of 14%, but does not need the addition of an egg yolk and to prevent microbial growth, antibiotics are also included in the extender (Rodger et al., 2009). Extended spermatozoa, allowed for a conception rate of 44%, which is only slightly lower than natural conception in zoos, with dilution of the spermatozoa causing no difference (Allen et al., 2008). Extended-chilled spermatozoa that has been chilled at 4 ^{o}C for 24-72 hours and is rewarmed in the female reproductive tract, has proven to be a sufficient amount of time to chill spermatozoa for transport without affecting the quality as fertility of the spermatozoa (Rodger et al. 2009, Allen et al. 2008). Using a tris-citrate buffer the spermatozoa has been known to survive at 5 ^{o}C for 42 days (Rodger et al., 2009). However, there has been a lack of evidence-based research on how the extender should be designed correctly. The use of dimethylamide can be used as an alternative to glycerol, allowing for 50%

of motility from the post thaw, which is high for when post-thaw survival in compromised once thawed and incubated at 35 ^{o} C, however the method needs to be improved on to achieve a higher conception rate (Rodger et al., 2009).

Unlike the koala, where chilled-extended semen is used, freshly extended semen is more common in elephants (Kiso et al., 2010). The optimal storage for liquid semen storage, with sperm viability being assessed included temperatures that are lower than the body temperature improves spermatozoa survival in elephants (Kiso et al., 2010). A source of lipoprotein, such as skim milk or an egg yolk help maintain Asian elephant spermatozoa quality better than other extenders while African elephant sperm tolerate all storage temperatures with either a tissue culture medium or an extender containing 20% egg yolk (Kiso et al., 2010). If the semen needs to be chilled, cryopreservation is the method, which is the same as the koalas; however, the design of the extender is different. Instead of 14% glycerol, 10% glycerol in the final volume of the extender is used to best preserve the post-thaw sperm motility, as well as the addition of a hen’s egg yolk, then later replaced with a Japanese quail egg yolk, when koalas did not need the addition of any egg yolk (Hermes et al., 2013). We also see that dilution in BC basic solution with 3%, 5% and 7% glycerol brings the elephant’s spermatozoa to a final concentration of approximately 250 – 300 x 10 ^{6} , something that had no effect on koala spermatozoa (Hermes et al., 2013). There are more steps that occur in freezing elephant spermatozoa than with koala spermatozoa, such as the semen sample is centrifuged at an ambient temperature of ~23 ^{o} C for 20 minutes at 1, 000g to remove seminal fluids

with samples underlain with 2mL of isothermal 60% iodixanol, another steps that does not occur when freezing koala spermatozoa (Hermes et al., 2013). By submerging the tubes in an isothermal water bath and then putting it in the fridge, the extenders were then cooled to 4 – 5 ^{o} C at ~0. 3 ^{o} C min $^{-1}$ (Hermes et al., 2013). Then, once chilled, the semen is then diluted even further to 150 x 10 ^{6} cells with a balance of 90% glycerol in the isothermal extenders (Hermes et al. 2013, Saragusty et al. 2009). Like koalas, the frozen samples are stored under liquid nitrogen in thin tubes (Hermes et al., 2013).

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