Int. Zoo Yb. (2003) 38: 160–172

Birth of Koalas

Phascolarctos cinereus

at Lone Pine Koala Sanctuary following artificial insemination

S. D. JOHNSTON[1], M. R. McGOWAN[1], P. O'CALLAGHAN[2], R. COX[3], B. HOULDEN[4], S. HAIG[2] & G. TADDEO[5]

[1]*School of Veterinary Science and Animal Production, The University of Queensland, 4072,* [2]*Lone Pine Koala Sanctuary, Jesmond Road, Fig Tree Pocket, Queensland 4069,* [3]*Bioquest Ltd, North Ryde, Sydney, New South Wales 2113,* [4]*Zoological Parks Board of New South Wales, PO Box 20, Mosman, New South Wales 2088 and School of Biological Science, The University of New South Wales, 2052,* and [5]*Cook Industries Australia Pty Ltd, Eight Mile Plains, Brisbane, Queensland 4122, Australia*

This paper documents the successful development of an artificial insemination (AI) programme for the Koala *Phascolarctos cinereus*. The protocols for trials involving two methods to induce ovulation and two insemination techniques are described. In Trial 1, interrupted coitus using a 'teaser' ♂ successfully induced ovulation in nine Koalas. Five ♀♀ were inseminated while conscious using a modified 'foley catheter' (Cook insemination catheter) resulting in the births of two offspring. The other four ♀♀ were anaesthetized and inseminated using a technique which allowed visualization of the most cranial portion of the urogenital sinus, where semen was deposited using a 3·5 Fr. 'Tom-cat catheter' (urogenitoscopic insemination). Three of the four ♀♀ inseminated by this technique produced pouch young. Microsatellite analysis of DNA from the pouch young excluded the teaser ♂♂ as possible sires, confirming that all offspring were sired by donor sperm. In Trial 2, eight ♀♀ were induced to ovulate by injecting them with 250 International Units of human chorionic gonadotrophin (hCG). A luteal phase was confirmed in all eight ♀♀ but only one gave birth following urogenitoscopic insemination. The Koala pouch young in this study are the first of any marsupial to be conceived and born following AI procedures. Details of the AI procedures used are presented and the significance of AI to the conservation biology of *P. cinereus* discussed.

Key-words: artificial insemination, assisted-breeding technology, conservation, induced ovulation, koala, marsupial

Assisted-breeding technology offers exciting new possibilities to enhance traditional methods of *ex situ* management and breeding of captive mammals (Holt, 1994). Although these techniques are increasingly being used in the captive-breeding of eutherian wildlife (Wildt *et al.*, 1993, 1997), the production of marsupial pouch young following artificial insemination (AI) has yet to be achieved (Mate *et al.*, 1998). The potential application of AI to the conservation and management of marsupial populations was originally proposed by Rodger (1990) and at time of writing there have been only three studies of AI in marsupials, as exemplified by the Brushtail possum *Trichosurus vulpecula* and Tammar wallaby *Macropus eugenii* (Molinia *et al.*, 1997, 1998; Nickel *et al.*, 1997). In combination with improved methods of superovulation, these researchers developed an intrauterine/intravaginal laparoscopic insemination system that resulted in fertilization. However, despite these significant advances, the production of pouch young has not been reported.

In May 1998, researchers at the University of Queensland and Lone Pine Koala Sanctuary (LPKS) announced the birth of a Koala *Phascolarctos cinereus* pouch young, produced following artificial induction of ovulation and insemination; a first for any species of marsupial.

Society of London

al

X[3],

Queensland,
ensland 4069,
Parks Board
School of
Industries
lia

nammals (Holt,
techniques are
in the captive-
wildlife (Wildt
duction of mar-
owing artificial
to be achieved
otential applica-
nservation and
ial populations
y Rodger (1990)
here have been
marsupials, as
ail possum *Tri-*
ammar wallaby
ia *et al.*, 1997,
In combination
of superovula-
eloped an intra-
oscopic insem-
ted in fertiliza-
hese significant
of pouch young

ers at the Uni-
nd Lone Pine
announced the
larctos cinereus
following artifi-
l and insemina-
s of marsupial.

Six months later, and following paternity analysis, a further five back-young were confirmed born following AI. This paper outlines the development and implementation of AI in Koala, describing the protocols and techniques employed in the successful production of offspring, as well as highlighting the potential applications of AI to Koala conservation.

DEVELOPMENT OF ARTIFICIAL INSEMINATION TECHNIQUES

Although the Koala is one of Australia's most recognizable native mammals, knowledge of the reproductive biology of the species was, until recently, limited. In an attempt to improve our basic knowledge and thereby facilitate the management of wild and captive Koala populations, a series of studies, critical to the development of an AI programme, was carried out (Johnston, 1999). Three key areas were investigated: (1) semen collection (Johnston *et al.*, 1994, 1997a) and preservation (Johnston *et al.*, 1992, 1993, 1998, 2000a), (2) detection of oestrus and the timing of ovulation (Johnston *et al.*, 2000b) and (3) anatomy of the ♀ reproductive tract to determine the most appropriate site for the deposition of semen (Johnston, 1999).

The use of AI in the Koala was first proposed by Biery (1991) while developing semen collection methods based on the use of an artificial vagina. Johnston *et al.* (1992) later suggested the use of AI when discussing the advantages of storing chilled (16°C) Koala semen for short-term preservation. In a review of the use of reproductive technology in the Koala, Johnston (1994) reported five unsuccessful preliminary attempts at AI. However, at that time it was not fully recognized that ovulation was induced by coitus (Johnston *et al.*, 1997b, 2000b,c). Given new information on the reproductive physiology, behaviour and anatomy of *P. cinereus*, a more targeted attempt at AI was possible.

Semen collection Since 1991 semen has been collected from Koalas by electroejaculation (Wildt *et al.*, 1991; Johnston *et al.*, 1994). However, this technique is comparatively invasive, requiring the ♂ to be placed under general anaesthesia and an electrode probe to be inserted in the rectum. In addition, some semen samples may coagulate following electroejaculation (Johnston *et al.*, 1994) making further manipulation of the semen for AI impossible. A breakthrough in the collection of semen from captive Koalas came with the development of a method that utilized an artificial vagina (Biery, 1991; Johnston *et al.*, 1997a). This technique was non-invasive and required no anaesthesia. Semen samples collected using an artificial vagina were typically larger in volume, had a higher sperm concentration and only rarely coagulated after ejaculation (Johnston *et al.*, 1997a).

Timing of insemination The detection of oestrus in the Koala is a comparatively simple procedure (Blanshard, 1994; Johnston *et al.*, 2000b) because the ♀ displays an overt behavioural oestrus (Smith, 1980). To detect oestrus, a ♂ 'teaser' Koala is placed in the ♀'s enclosure where he will characteristically scent tree poles with his sternal gland, urinate and then start to bellow. This behaviour alerts ♀♀ to the presence of the ♂ and ♀♀ that are sexually receptive start to display typical oestrous behaviour.

The most conspicuous ♀ behaviour is 'jerking' or 'convulsive' behaviour where the ♀ appears to be 'hiccoughing' uncontrollably. Oestrous ♀♀ may also attempt to mate with other oestrous ♀♀ in the enclosure (Johnston *et al.*, 2000b). Other oestrous behaviours include restlessness, in which the ♀ may pace around the enclosure, bellowing and urinating in response to the presence of the ♂. Oestrus in non-mated Koalas usually lasts *c.* 10 days (mean ± SE 10·3 ± 0·9 days; range 0–19 days) (Johnston *et al.*, 2000b).

A significant advance in the development of the Koala AI programme was the discovery that ovulation appears to be induced by coitus (Johnston *et al.*, 1997b, 2000b,c). Previous estimates of the length of the oestrous cycle in *P. cinereus* (*c.* 28–35 days) had presumably been based on non-mated anovulatory cycles (Brown, 1987; Handasyde *et al.*, 1990); further investigation showed that the actual length of an oestrous cycle incorporating a luteal phase was significantly longer at 49·5 ± 1·0 days (Johnston *et al.*, 2000b).

Recent studies have also shown that it is possible to induce a luteal phase in Koala in preparation for AI by means of interrupted coitus, where the penis of the teaser ♂ is diverted out of the ♀'s urogenital sinus before ejaculation of the sperm-rich fraction (Johnston *et al.*, 2000c). However, because there is a 12·5% probability that semen from the teaser ♂ may be emitted prematurely during interrupted coitus, genetic analysis is required to determine paternity of any resulting offspring.

When eight ♀♀ were injected with 250 International Units (IU) of human chorionic gonadotrophin (hCG) (Chorulon®), a luteal phase was successfully induced in all of them but it was not possible to determine conclusively whether the ♀♀ had ovulated (Johnston *et al.*, 2000c).

Placement of the semen The reproductive tract of ♀ Koalas, like all marsupials, comprises two lateral vaginae (Johnston, 1999). This anatomy makes the placement of semen directly into the uterus via the lateral vaginae or vaginal culs-de-sac virtually impossible unless a laparoscopic insemination protocol is used. The Koala also has a septum of tissue separating the left and right sides of the vaginal culs-de-sac; therefore, semen may need to be deposited into both lateral vaginae.

Catheter design The design of a catheter suitable for depositing semen into the upper urogenital sinus was based on measurements of the Koala reproductive tract in the proliferate phase of the oestrous cycle (Johnston, 1999) and a cast of the ♀ urogenital canal. The cast was made from the lower reproductive tract of a sexually mature ♀ Koala cadaver, by introducing *c.* 5 ml liquid epoxy resin into the urogenital sinus. Based on these observations, Cook Industries Australia Pty Ltd, Brisbane, helped to develop a prototype Cook insemination catheter; essentially a customized 'foley catheter' (Plate 1). Designed to be inserted *c.* 25 30 mm into the ♀'s urogenital sinus, the catheter has a cuff situated 10 mm behind the tip which can be inflated to a diameter of 13 mm. When the inflated cuff is held firmly in place against the inner margins of the urogenital sinus, semen deposited in front of the cuff can be displaced into the dorsocranial portion of the urogenital sinus and into both lateral vaginae.

ARTIFICIAL INSEMINATION TRIALS

Animals Following preliminary investigations, between December 1997 and March 1998 AI was carried out on 17 sexually mature ♀ Koalas maintained at LPKS. Twenty sexually mature ♂♂ were used either as teaser ♂♂ to induce ovulation or as sperm donors. The Koalas were all clinically healthy throughout the trial period and the husbandry of Koalas at LPKS has been described previously (Blanshard, 1994). The breeding records at LPKS were consulted so that, wherever possible, the donor sire and the dam were chosen to maximize the likelihood of heterozygosity in the offspring, thereby facilitating subsequent paternity analysis of pouch young.

Semen collection and characteristics Semen was collected from 14 ♂♂ using an artificial vagina (Johnston *et al.*, 1997a) and semen characteristics, including percentage of forwardly motile spermat-

ign of a catheter
semen into the
was based on
ala reproductive
hase of the oes-
99) and a cast of
ıe cast was made
ve tract of a sex-
daver, by intro-
xy resin into the
n these observa-
ustralia Pty Ltd,
lop a prototype
ter; essentially a
ıeter' (Plate 1).
. 25 30 mm into
ıe catheter has a
nd the tip which
neter of 13 mm.
s held firmly in
ırgins of the uro-
ısited in front of
into the dorso-
genital sinus and

N TRIALS
iminary investi-
nber 1997 and
ed out on 17 sex-
maintained at
mature ♂♂ were
to induce ovula-
The Koalas were
ughout the trial
ry of Koalas at
ibed previously
ıreeding records
o that, wherever
ıd the dam were
e likelihood of
fspring, thereby
aternity analysis

characteristics
ı 14 ♂♂ using an
ın *et al.*, 1997a)
ıtics, including
motile spermat-

ozoa (%FM), rate of sperm movement (R) and sperm concentration, were evaluated (Johnston *et al.*, 1997a). The undiluted semen sample was maintained at room temperature (22–25°C) for *c.* 15–30 minutes prior to insemination.

Trial 1: ovulation induced by interrupted coitus Ovulation was induced in nine oestrous ♀♀ by allowing a teaser ♂ to mount the ♀, achieve intromission and reach the end of his thrusting period (Johnston, 2000c). Details of the copulatory behaviour of the teaser ♂ were recorded. The ♂ was then removed and the ♀'s urogenital sinus was swabbed to determine the presence of sperm.

Two methods of insemination were attempted. The first used a modified foley catheter, or Cook insemination catheter, as described above. Approximately 30 minutes after interrupted coitus, the conscious oestrous ♀ was gently restrained in a partial upright position, termed insemination recumbency (Plate 2). Approximately 1 ml undiluted semen was drawn up into a 1 ml syringe. The catheter was then inserted *c.* 25 mm into the urogenital sinus and approximately positioned so that the semen would be deposited into the dorsal portion of the urogenital sinus, immediately adjacent to the openings of the lateral vaginae. The cuff of the insemination catheter was then inflated using *c.* 1·3 ml sterile water and gently retracted to ensure that the cuff was securely in place. The 1 ml syringe was then attached to the catheter and semen deposited slowly into the urogenital sinus, followed by 1 ml of air. The ♀ was maintained in insemination recumbency for a further 15 minutes before the cuff was deflated and the catheter removed. Five ♀♀ were inseminated using this method.

The remaining four ♀♀ were inseminated using a urogenitoscopic approach. Approximately 30 minutes after interrupted coitus, the ♀ was placed under light gaseous, Isoflurane anaesthesia (Mc-

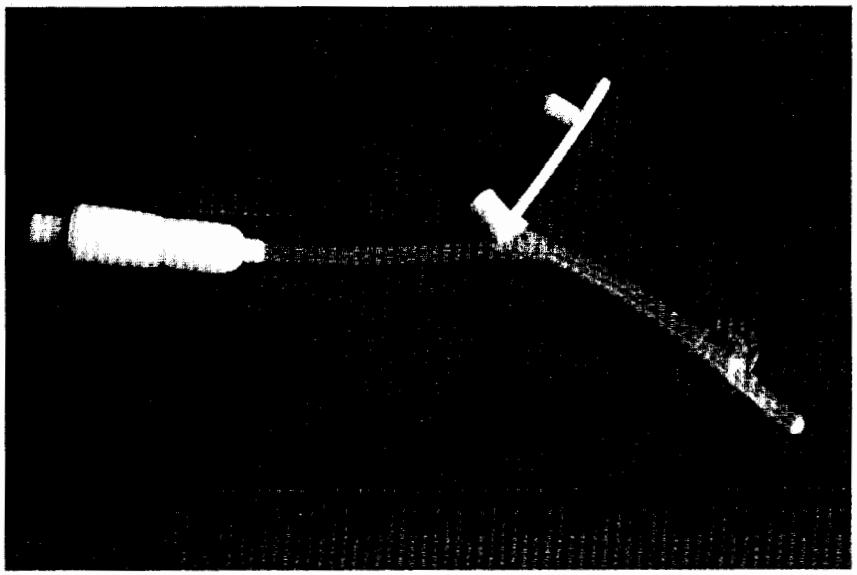

Plate 1. Modified foley (Cook) insemination catheter developed for artificial insemination procedures in Koala *Phascolarctos cinereus*. Cook Australia Pty Ltd.

ign of a catheter
semen into the
was based on
ala reproductive
hase of the oes-
99) and a cast of
ie cast was made
ve tract of a sex-
daver, by intro-
ky resin into the
n these observa-
ustralia Pty Ltd,
lop a prototype
ter; essentially a
ieter' (Plate 1).
. 25–30 mm into
he catheter has a
nd the tip which
neter of 13 mm.
s held firmly in
irgins of the uro-
sited in front of
into the dorso-
genital sinus and

N TRIALS
iminary investi-
nber 1997 and
id out on 17 sex-
maintained at
nature ♂♂ were
to induce ovula-
The Koalas were
ughout the trial
ry of Koalas at
ibed previously
breeding records
o that, wherever
nd the dam were
e likelihood of
fspring, thereby
aternity analysis

characteristics
14 ♂♂ using an
n *et al.*, 1997a)
itics, including
motile spermat-

ozoa (%FM), rate of sperm movement (R) and sperm concentration, were evaluated (Johnston *et al.*, 1997a). The undiluted semen sample was maintained at room temperature (22–25°C) for *c.* 15–30 minutes prior to insemination.

Trial 1: ovulation induced by interrupted coitus Ovulation was induced in nine oestrous ♀♀ by allowing a teaser ♂ to mount the ♀, achieve intromission and reach the end of his thrusting period (Johnston, 2000c). Details of the copulatory behaviour of the teaser ♂ were recorded. The ♂ was then removed and the ♀'s urogenital sinus was swabbed to determine the presence of sperm.

Two methods of insemination were attempted. The first used a modified foley catheter, or Cook insemination catheter, as described above. Approximately 30 minutes after interrupted coitus, the conscious oestrous ♀ was gently restrained in a partial upright position, termed insemination recumbency (Plate 2). Approximately 1 ml undiluted semen was drawn up into a 1 ml syringe. The catheter was then inserted *c.* 25 mm into the urogenital sinus and approximately positioned so that the semen would be deposited into the dorsal portion of the urogenital sinus, immediately adjacent to the openings of the lateral vaginae. The cuff of the insemination catheter was then inflated using *c.* 1·3 ml sterile water and gently retracted to ensure that the cuff was securely in place. The 1 ml syringe was then attached to the catheter and semen deposited slowly into the urogenital sinus, followed by 1 ml of air. The ♀ was maintained in insemination recumbency for a further 15 minutes before the cuff was deflated and the catheter removed. Five ♀♀ were inseminated using this method.

The remaining four ♀♀ were inseminated using a urogenitoscopic approach. Approximately 30 minutes after interrupted coitus, the ♀ was placed under light gaseous, Isoflurane anaesthesia (Mc-

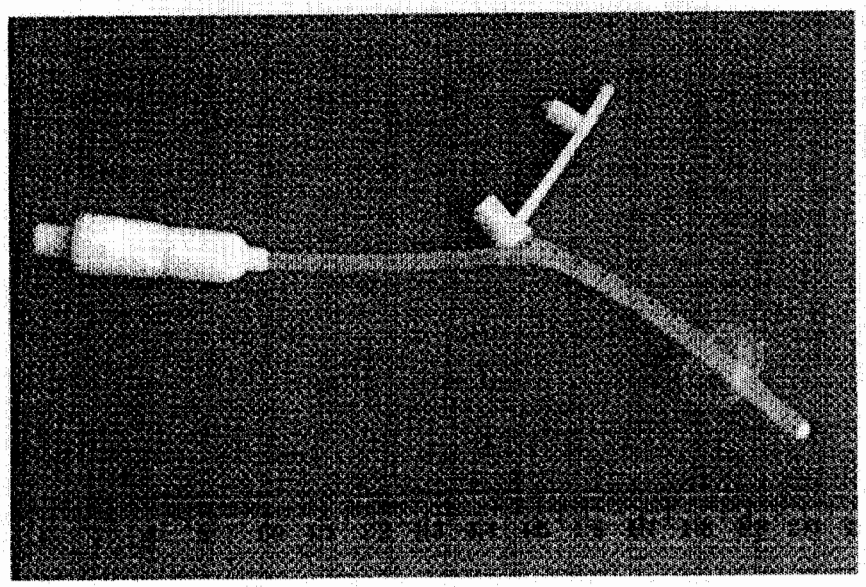

Plate 1. Modified foley (Cook) insemination catheter developed for artificial insemination procedures in Koala *Phascolarctos cinereus. Cook Australia Pty Ltd.*

Gowan *et al.*, 1995) and positioned in ventral recumbency on a tilting table set at an incline of *c.* 30° with the hind quarters extended over the edge of the table. An otoscope with a 70 mm-long speculum was inserted into the urogenital sinus to visualize the longitudinal grooves extending towards the entrances of the left and right lateral vaginae. To ensure that all the semen in the catheter was expelled, 0·5 ml air was initially drawn up, followed by *c.* 0·5–0·6 ml semen, into a 3·5 Fr. 'Tom-cat catheter', connected to a 1 ml syringe. The Tom-cat catheter was carefully positioned down the barrel of the speculum and along the longitudinal grooves of the urogenital sinus (Johnston, 1999). Given the extreme hyperplasia and hypertrophy of the epithelium lining the lumen of the urogenital sinus (Johnston, 1999), it was often not possible to be certain of insemination directly into the lumen of each lateral vagina. In these circumstances, semen was deposited into the most cranial portion of the left and right longitudinal grooves of the urogenital sinus, immediately adjacent to the lateral vaginal ostia. After insemination, ♀♀ remained in elevated ventral recumbency for a further 5 minutes before being allowed to recover from anaesthesia in lat-

Plate 2. Artificial insemination of a conscious Koala using the Cook insemination catheter. *S. D. Johnston.*

eral recumbenc
subsequently isc

Trial 2: ovula
Induction of ov
a further eight l
hCG (Chorulor
pinatus muscle
ston *et al.*, 2000
using the Coc
method and sev
the urogenitosc

Progestogen ass
luteal phase B
from the ♀♀ in
plasma progestc
day of insemina
28 days after ir
injection. Prog
have been descr
et al., 2000b).
luteal phase in r
confirmed by a
gestogen conce
days 14 and 28
result in a birth
ularly for signs
oestrous interv
for the presence
were carried
insemination.

Paternity analy
lected from adu
tubes containin
and frozen at
genetic analysis
biopsy was tak
the right flank
lowing AI v
6–7 months old.
the biopsies an
out using five h
markers, as
(Houlden *et al*
assigned by exc

Results of Trial
thrusting beha

Gowan *et al.*, 1995) and positioned in ventral recumbency on a tilting table set at an incline of *c.* 30° with the hind quarters extended over the edge of the table. An otoscope with a 70 mm-long speculum was inserted into the urogenital sinus to visualize the longitudinal grooves extending towards the entrances of the left and right lateral vaginae. To ensure that all the semen in the catheter was expelled, 0·5 ml air was initially drawn up, followed by *c.* 0·5–0·6 ml semen, into a 3·5 Fr. 'Tom-cat catheter', connected to a 1 ml syringe. The Tom-cat catheter was carefully positioned down the barrel of the speculum and along the longitudinal grooves of the urogenital sinus (Johnston, 1999). Given the extreme hyperplasia and hypertrophy of the epithelium lining the lumen of the urogenital sinus (Johnston, 1999), it was often not possible to be certain of insemination directly into the lumen of each lateral vagina. In these circumstances, semen was deposited into the most cranial portion of the left and right longitudinal grooves of the urogenital sinus, immediately adjacent to the lateral vaginal ostia. After insemination, ♀♀ remained in elevated ventral recumbency for a further 5 minutes before being allowed to recover from anaesthesia in lat-

Plate 2. Artificial insemination of a conscious Koala using the Cook insemination catheter. *S. D. Johnston.*

eral recumbenc
subsequently isc

Trial 2: ovula
Induction of ov
a further eight I
hCG (Chorulor
pinatus muscle
ston *et al.*, 2000
using the Coc
method and sev
the urogenitosc

Progestogen ass
luteal phase B
from the ♀♀ in
plasma progest
day of insemina
28 days after it
injection. Prog
have been descr
et al., 2000b).
luteal phase in
confirmed by a
gestogen conce
days 14 and 28
result in a birth
ularly for signs
oestrous interv
for the presence
were carried
insemination.

Paternity analy
lected from adu
tubes containin
and frozen at
genetic analysis
biopsy was tak
the right flank
lowing AI v
6–7 months old
the biopsies an
out using five h
markers, as
(Houlden *et al*
assigned by exc

Results of Trial
thrusting beha

sinus (Johnston, hyperplasia and elium lining the sinus (Johnston, ossible to be cer- rectly into the ina. In these cir- eposited into the he left and right the urogenital nt to the lateral semination, ♀♀ tral recumbency s before being naesthesia in lat-

eral recumbency. Inseminated ♀♀ were subsequently isolated from ♂ contact.

Trial 2: ovulation induced by hCG Induction of ovulation was attempted in a further eight Koalas by injecting 250 IU hCG (Chorulon®) into the right supraspinatus muscle on day 2 of oestrus (Johnston *et al.*, 2000c). One ♀ was inseminated using the Cook insemination catheter method and seven were inseminated using the urogenitoscopic procedure.

Progestogen assay and confirmation of a luteal phase Blood (2 ml) was collected from the ♀♀ in Trials 1 and 2 to measure plasma progestogen concentrations on the day of insemination (day 0) and at 14 and 28 days after interrupted coitus or hCG injection. Progestogen assay procedures have been described previously (Johnston *et al.*, 2000b). Successful induction of a luteal phase in non-parturient Koalas was confirmed by a significantly elevated progestogen concentration (>0·9 ng/ml) on days 14 and 28. If insemination did not result in a birth, the ♀♀ were checked regularly for signs of oestrus and the interoestrous interval recorded. Daily checks for the presence of offspring in the pouch were carried out from day 33 after insemination.

Paternity analysis Blood (4 ml) was collected from adult Koalas into vacutainer tubes containing 100 µl 15% K_3-EDTA and frozen at −20°C for subsequent genetic analysis. A 3 mm-diameter tissue biopsy was taken from the loose skin of the right flank of all Koalas born following AI when the young were 6–7 months old. DNA was extracted from the biopsies and paternity testing carried out using five hypervariable microsatellite markers, as described previously (Houlden *et al.*, 1996). Paternity was assigned by exclusion analysis.

Results of Trial 1 The characteristics of thrusting behaviour and incidence of premature ejaculation by teaser ♂♂ used to induce ovulation in nine Koalas in Trial 1 are presented in Table 1. The mean (±SE) number of thrusts and period of thrusting was 35·8±3·1 and 20·4±1·2 seconds, respectively. Spermatozoa were detected in the urogenital sinus of three of the nine ♀♀ following thrusting. Table 1 also records the characteristics of the donor semen. The mean (±SE) sperm cell concentration, %FM and R were 162·2±33·9 million/ml, 76·1±2·6 and 3·9±0·2, respectively.

The upper limit of the 99·9% confidence interval of progestogen concentration in blood taken during oestrus from all 17 Koalas (Trails 1 and 2) was 0·9 ng/ml. The progestogen concentration in samples collected on days 14 and 28 after interrupted coitus are shown in Table 1 and confirm that a luteal phase was successfully induced in all nine ♀♀. The mean (±SE) progestogen concentration on days 14 and 28 in pregnant Koalas was 6·2±1·2 ng/ml and 15·7±3·3 ng/ml, respectively, while the progestogen concentration on the same days in non-parturient Koalas (F8, F42, F43, F36) was 5·0±0·7 ng/ml and 10·9±1·4 ng/ml. Three of the four ♀♀ which failed to give birth displayed signs of oestrus *c.* 52·7±1·2 days after coitus. Five of the nine ♀♀ (F20, F44, F2, F28, F45) which were artificially inseminated gave birth to live pouch young.

Microsatellite genotyping at five variable loci and simple exclusion analysis were carried out to determine paternity of the pouch young (Table 2). Microsatellite typing of the five dam–offspring dyads was consistent with known relationships based on observations made by the keeper. Triad relationships were then investigated and the teaser ♂♂ could be excluded as potential sires for at least two of the five loci scored. Therefore paternity of all five offspring could unambiguously be assigned to the donor ♂.

	C					T			
	F8	F20	F42	F43	F44	F36	F2	F28	F45
Teaser ♂	M18	M19	M12	M33	M19	M30	M36	M23	M36
No. thrusts/	41/	42/	47/	37/	27/	25/	42/	30/	32/
length of thrusting period (seconds)	27	25	20	20	17	20	20	15	20
Sperm of teaser ♂ present	no	no	no	no	no	yes	no	yes	yes
Donor ♂	M31	M31	M15	M34	M32	M35	M37	M24	M30
Sperm concentration (millions/ml)	140	120	150	400	120	200	200	80	50
Forward motility (%)	65	70	70	80	90	80	80	70	80
Rate of sperm movement (0–5)	4	3·5	4	5	4	4	4	3	3·5
Volume of inseminate (ml)	0·7	1	1·1	1	0·8	0·9	1	1·2	0·9
PROGESTOGEN ANALYSIS									
day 0 (ng/ml)	0·5	1	0·2	0·3	0·3	0·9	0·1	0·1	0·5
day 14 (ng/ml)	6·4	4·9	3·1	4·9	9·4	5·4	3·4	8·7	4·8
day 28 (ng/ml)	13·3	9·8	12	7·1	26	11·2	20·8	11·9	10
Days to next oestrus after coitus	55	birth	ND	51	birth	52	birth	birth	birth
Paternity analysis excluded teaser ♂	NA	yes	NA	NA	yes	NA	yes	yes	yes

Table 1. Summary of Koala *Phascolarctos cinereus* artificial insemination Trial 1 in which ♀♀ were induced to ovulate using interrupted coitus: C. Cook insemination catheter procedure; T. Tom-cat catheter urogenitoscopic procedure; ND. no data; NA. not applicable.

Results of Trial 2 The characteristics of the eight donor ejaculates used in AI and the progestogen assays results from Trial 2 are shown in Table 3. The mean (±SE) sperm cell concentration, %FM and R were 257·5±31·5 million/ml, 74·0±3·6 and 3·8±0·1, respectively.

Hormone analysis of blood samples collected on days 14 and 28 confirms that a luteal phase occurred in all eight ♀♀ induced to ovulate by hCG injection. The mean (±SE) progestogen concentration on days 14 and 28 was 7·1±0·7 ng/ml and 18·3±4·2 ng/ml, respectively, which is not significantly different from progestogen concentrations in ♀♀ induced to ovulate using interrupted coitus. Artificial insemination resulted in only one birth, by F48, following the urogenitoscopic procedure.

During the insemination of ♀♀ F49 and F54, most of the inseminate coagulated in the catheter, therefore both ♀♀ failed to receive the full inseminate. Koala F51 urinated during the insemination procedure, potentially contaminating the inseminate and possibly rendering it infertile.

Comparison of methods The results of Trial 1 show that AI in Koalas using interrupted coitus to induce ovulation resulted in five of the nine ♀♀ becoming pregnant. All pouch young were conceived from donor semen, indicating that even though spermatozoa from the teaser ♂ were present in the urogenital sinus of some ♀♀ after interrupted coitus, the number of sperm was probably not sufficient to result in fertilization. One ♀ (F45) gave birth following insemination with semen which had a sperm count of only 50 million/ml, approximately one-third of the mean ejaculate sperm concentration recorded by Johnston *et al.* (1997a). This result suggests that it may be possible to dilute Koala semen, thereby permitting the insemination of more than one ♀ from one donor ♂. Further experience in AI is required before the relationships between the various characteristics of the ejaculate and the successful production of pouch young can be elucidated fully.

Similarly, while the urogenitoscopic procedure resulted in a slightly higher

2	F28	F45
136	M23	M36
42/	30/	32/
20	15	20
no	yes	yes
137	M24	M30
200	80	50
80	70	80
4	3	3·5
1	1·2	0·9
0·1	0·1	0·5
3·4	8·7	4·8
20·8	11·9	10
rth	birth	birth
yes	yes	yes

h ♀♀ were induced to heter urogenitoscopic

The results of
n Koalas using
nduce ovulation
ne ♀♀ becoming
ɔung were con-
ı, indicating that
ı from the teaser
ogenital sinus of
ɔted coitus, the
obably not suffi-
ion. One ♀ (F45)
semination with
m count of only
.tely one-third of
m concentration
al. (1997a). This
ıy be possible to
ɛreby permitting
than one ♀ from
perience in AI is
ionships between
s of the ejaculate
uction of pouch
fully.
urogenitoscopic
ı slightly higher

pregnancy rate than the Cook insemination catheter method in Trial 1, further AI trials will be required before one method can be considered superior to the other. The Cook insemination catheter method is preferred because neither anaesthesia nor the use of an otoscope to visualize the entrances of the lateral vaginae are required. The Koala pouch young born in Trial 1 are the first marsupials to be conceived and born following AI.

Although eight ♀♀ were induced to ovulate by injecting hCG, only one ♀ gave birth. However, the resulting pouch young was the first marsupial to be born following hormonal induction of ovulation together with AI. The lack of success in the remaining ♀♀ requires further investigation but was most likely related to the failure of either ovulation or fertilization. In Trial 2 it was not possible to determine directly whether hCG administration had resulted in ovulation, or whether ovulation had occurred but the oocytes had become trapped within luteinized follicles.

ROLE OF AI IN KOALA CONSERVATION

A recent review of the status of *P. cinereus* in Australia (ANZECC, 1998) revealed that while the species was under no immediate threat of extinction on a national basis, regional conservation status varied significantly, being secure in some areas and vulnerable or extinct in others. For example, in Queensland, population numbers are declining slowly and in New South Wales Koalas have disappeared from 50–75% of their range as a result of habitat loss. In contrast, some populations in Victoria and South Australia occur at such high densities that they are causing significant defoliation of eucalypt

INDIVIDUAL	RELATIONSHIP	GENOTYPE OF ALLELES AT LOCUS				
		Phci-2	Phci-25	Phci-11	Phci-1	Phci-4
F20	Dam	172/172	125/123	163/161	118/100	111/109
P1F20	Pouch young	190/172	123/123	169/161	116/100	111/111
M19*	Teaser	180/172	147/129	169/159	102/100	111/109
M31	Donor	194/190	123/123	179/169	116/102	111/109
F44	Dam	180/172	155/155	169/169	118/118	111/109
P2F44	Pouch young	172/166	155/125	169/159	118/100	111/109
M19*	Teaser	180/172	147/129	169/159	102/100	111/109
M32	Donor	172/166	149/125	163/159	102/100	111/109
F2	Dam	192/172	149/125	185/179	118/100	109/109
P3F2	Pouch young	172/166	155/125	179/169	118/118	111/109
M36*	Teaser	196/172	155/131	169/163	118/118	119/109
M37	Donor	172/166	155/131	169/159	118/102	111/109
F28	Dam	196/172	133/129	169/161	100/100	111/111
P4F28	Pouch young	196/196	147/133	169/157	102/100	119/111
M23*	Teaser	192/192	155/125	179/163	118/104	109/107
M24	Donor	196/172	147/123	169/157	102/100	119/119
F45	Dam	196/192	147/125	161/159	102/100	111/109
P5F45	Pouch young	196/190	147/135	161/159	102/100	111/109
M36*	Teaser	196/172	155/131	169/163	118/118	119/109
M30	Donor	196/190	135/133	163/161	100/100	111/109

* Males excluded as sires at two or more loci.

Table 2. Paternity assignment in Koalas born by artificial insemination. Genotypes are listed as allele size relative to a standard size marker, as described previously (Houlden *et al.*, 1996).

	C	T						
	F49*	F48	F49	F50	F51**	F52	F53	F54*
Donor ♂	M24	M34	M49	M40	M41	M42	M43	M44
Sperm concentration (millions/ml)	100	200	240	220	300	400	300	300
Forward motility (%)	60	80	84	86	83	68	64	67
Rate of sperm movement (0–5)	3	4	4	4	4	4	3·5	4
Volume of inseminate (ml)	0·8	0·6	1·3	1·6	1·1	1	0·6	1
PROGESTOGEN ANALYSIS								
day 0 (ng/ml)	0·5	0·2	1·6	1·0	1·1	1·0	0·3	0·2
day 14 (ng/ml)	5·3	7·5	5·4	7·8	9·5	5·3	5·7	10·2
day 28 (ng/ml)	6·8	15·5	6·6	42·8	15·8	13·5	23·3	22·1
Birth	no	yes	no	no	no	no	no	no

Table 3. Summary of Koala artificial insemination Trial 2 in which ♀♀ were induced to ovulate using 250 IU hCG: C. Cook insemination catheter procedure; T. Tom-cat catheter urogenitoscopic procedure; * semen coagulated in insemination catheter; ** ♀ urinated during insemination.

Eucalyptus spp, prompting recommendations to cull animals (Possingham *et al.*, 1996). *Phascolarctos cinereus* is classified as Lower Risk (near threatened) by IUCN (Hilton-Taylor, 2000).

Koalas appear to reproduce successfully in the wild and in captivity. Martin & Handasyde (1990) reported that the intrinsic rate of increase of some wild disease-free Koala populations in Victoria can be as high as 0·26, which is approximately equivalent to a doubling of the population every 3 years. At time of writing LPKS maintains *c.* 130 Koalas, the majority of which were born at the Sanctuary. A selective-breeding programme, managed to maintain maximum genetic diversity of the captive population, has resulted in the birth of *c.* 30 pouch young each breeding season (O'Callaghan, 1996).

Given that *P. cinereus* is not currently threatened with extinction and that the species appears to have little difficulty reproducing, there would seem to be no great advantage in attempting to improve reproductive output via assisted reproduction techniques. However, the development of artificial-breeding technology in *P. cinereus* will be advantageous in the longer term.

Understanding basic reproductive biology Reproduction is a key variable in ecological models of population growth. Knowledge of the reproductive physiology and behaviour of the Koala gained through the development of an artificial-breeding programme will greatly influence the strategies and implementation of management plans for conservation of the species. O'Callaghan (1996) reported that the mean natural birth rate of Koalas maintained at LPKS from 1988 to 1996 was 57% but that in some seasons the rate was as low as 43%. A better understanding of the fundamentals of Koala reproduction is likely to improve further the breeding potential and reproductive management of captive populations. Alternatively, Possingham *et al.* (1996) recommended using methods of fertility suppression for controlling Koala population numbers in South Australia. The development and application of such techniques can only progress if normal reproductive function in the Koala is understood.

Reproductive tools for genetic management The cryopreservation of Koala semen, in combination with an AI programme, has the potential to facilitate

F53	F54*
M43	M44
300	300
64	67
3·5	4
0·6	1
0·3	0·2
5·7	10·2
23·3	22·1
no	no

e using 250 IU hCG: semen coagulated in

reproductive a key variable of population he reproductive r of the Koala elopment of an nme will greatly nd implementa- is for conserva- allaghan (1996) atural birth rate PKS from 1988 in some seasons 43%. A better ndamentals of kely to improve ntial and repro- captive popu- ssingham *et al.* ng methods of ntrolling Koala outh Australia. plication of such gress if normal the Koala is

for genetic preservation of tion with an AI ntial to facilitate

the genetic exchange required to maintain high levels of heterozygosity and evolutionary potential. Genetic diversity of captive populations, within Australia and overseas, could be maintained and improved by utilizing spermatozoa collected from unrelated free-ranging ♂♂. Semen can be collected safely from Koalas in the field, frozen and transported, thereby eliminating the need to translocate the animals.

The use of AI may also directly benefit wild Koala populations. As free-ranging populations continue to be subjected to fragmentation and genetic isolation as a result of habitat loss and degradation, AI could be used to introduce new genotypes from other wild populations or from well-managed captive colonies, for example, LPKS. The technology is now available for the collection and cryo-banking of semen from every Koala population in Australia (Johnston *et al.*, 1993, 1994, 1997a). Such an undertaking would potentially secure a high proportion of the existing heterozygosity for the long term.

Transport of frozen semen One of the initial reasons for developing an AI programme for the Koala (Johnston *et al.*, 1992) was to respond to the adverse public reaction in Australia over the shipment of live Koalas to overseas zoological institutions. At time of writing there are 26.32 Koalas in zoos in the USA, and 32.47 in Japan (ANZECC, 1998). The ability to transport frozen semen would eliminate the need to ship ♂ Koalas overseas. Semen from 50 Koalas, slowly introduced into a small captive population overseas through AI, would impart enough genetic vigour to maintain sufficient heterozygosity for decades, perhaps centuries. The transport of semen rather than live animals would also be significantly less expensive: shipment of frozen semen requires minimal specialized care or cargo arrangements, and allows for the transport of genetic material from a greater number of founders at any one time. At time of writing there are no specific quarantine or legal ownership provisions related to the shipping of marsupial semen overseas. However, it is likely that the quarantine regulations used for the transit of semen from livestock or other non-domestic animals could easily be modified to apply to Koala semen.

Monitoring or overcoming infertility Artificial reproduction techniques are extremely useful for identifying and overcoming specific anatomical, physiological and behavioural infertility problems. For example, it may be possible to identify infertility in the ♂ Koala by determining the characteristics of the ejaculate and the proportion of abnormal sperm, as has been achieved for many domestic animals (Barth & Oko, 1986; Hafez, 1993). Or, if a genetically important sire is unable to mate with a chosen ♀ because of injury, incapacitation or poor libido (Biery, 1991), semen can be collected by electroejaculation and the selected ♀ subsequently inseminated. Johnston (1994) has demonstrated that the characteristics of the electroejaculate from blind and/or disabled Koalas were not dissimilar to those of other healthy Koalas. Motile spermatozoa can also be recovered from the cauda epididymides of necropsied specimens up to 24 hours *post mortem* (S. D. Johnston, pers. obs), cryopreserved and potentially used at a later stage in an AI programme.

Preliminary studies have shown that *Chlamydia* sp can be detected in Koala seminal plasma (P. Timms & S. D. Johnston, unpubl. data); it may be possible to screen Koala semen for *Chlamydia* sp or other infectious agents before use in AI. It may also be possible to treat the semen with appropriate antibiotics in order to remove pathogenic organisms before use. These examples highlight the possibilities of introducing genetic diversity from valuable ♂♂ that may otherwise not be able to contribute to the gene pool. These approaches may be particularly important for small wildlife parks or over-

seas zoos which do not have ready access to alternative sires.

The Koala as a research model The research carried out to date demonstrates that *P. cinereus* is an excellent model species for reproductive studies (Johnston, 1994). Their comparatively passive nature, moderate size and habituation to captivity make handling the animals, the collection of blood and reproductive examinations straightforward procedures requiring no sedation or anaesthesia. Even for comparatively invasive procedures Koalas show good tolerance to injectable and gaseous anaesthesia (Blyde, 1990; Bush *et al.*, 1990; McGowan *et al.*, 1995). Repeated electroejaculation (Johnston *et al.*, 1994) and chronic catheterization (Cleva *et al.*, 1994) do not appear to have any obvious ill effects.

The ♀ Koala displays an overt behavioural oestrus (Blanshard, 1994), making detection of oestrus unambiguous. Large parks in south-east Queensland, such as LPKS and Dreamworld, use similar systems to those used at a commercial piggery to manage Koalas and for breeding husbandry (Blanshard, 1994), providing excellent opportunities for reproductive studies.

Like the Brushtail possum, Tammar wallaby and Fat-tailed dunnart *Sminthopsis crassicaudata*, the Koala may prove to be a useful reproductive model (Wildt *et al.*, 1986) for the development of artificial-breeding technology in other marsupials, particularly the closely related wombat species; for example, the Critically Endangered Northern hairy-nosed wombat *Lasiorhinus krefftii* (Hilton-Taylor, 2000).

A proactive approach A major problem with many conservation programmes is that, by the time a species is threatened with extinction, population numbers are so low that research into basic reproductive physiology and anatomy is difficult and perhaps even detrimental to survival of the remaining individuals. Such a crisis situation is generally not appropriate for the development and application of assisted reproductive technology, and is one of the primary reasons for its general lack of success.

A proactive approach to understanding the reproductive biology of the species is therefore required while the population is still comparatively large and in a reasonably strong ecological position. Given that significant numbers of Koalas still survive in the wild (ANZECC, 1998) and that they breed well in captivity (Blanshard, 1994), *P. cinereus* is a species that could benefit from the proactive development of artificial-breeding technology. The successful development and long-term application of AI will be a useful management tool to help ensure survival of the Koala far into the future.

The development of assisted-breeding technology leading to successful AI in the Koala, as reported here, has not only contributed substantially to a better understanding of the fundamental reproductive biology of this species but also should enhance our ability to manage and conserve *P. cinereus* both in the wild and in captivity. Most important, these studies have demonstrated that artificial breeding of marsupials is possible and, with further research, similar technology may also be developed to improve the reproductive potential of other more highly threatened species, such as the Northern hairy-nosed wombat.

ACKNOWLEDGMENTS

The authors would like to thank Tom Sakurabu, General Manager of Lone Pine Koala Sanctuary at time of writing, and Toshi Akiyama, former General Manager, for allowing access to facilities and collaboration with staff. In particular, we thank Jacque Brumm and Karen Nilsson who helped with the procedures to detect oestrus and collected Koala blood samples. We would also like to acknowledge the generous support of Jim Kelly, Bioquest Ltd, North Ryde, Sydney, for providing the laboratory space and equipment to run hormone assays. We also thank staff of the Queensland National Parks and Wildlife Service Koala Hospital, Moggill, for allowing access to
reproductive anator
Dr F. N. Carrick f
the manuscript. Th
was supported by
Koala Sanctuary an

PRODUCTS MENTIONI

Chorulon®: hCG
Intervet Australia
Road, Castle-Hill, N
Isoflurane: gaseous
Abbott Australasia
Kurnell, NSW 2231
Modified foley inse
manufactured by C
12 Electronics Stree
Australia.
Otoscope: Welch A
head with specula,
by Welch Allyn Pt
PO Box 220, Skar
USA.
Plastibond: liquid
Selley's Pty Ltd, 1/
4010, Australia.
Tom-cat catheter: M
by Becton Dickins
Drive, North Ryde.
Vacutainer tubes: E
manufactured by
Research Park Dr
Australia.

REFERENCES

ANZECC (1998): *strategy*. Canberra:
BARTH, A. D. & O
phology of bovine
University Press.
BIERY, K. (1991): S
64(8): 12–13.
BLANSHARD, W. H.
of koalas. In *Unive*
mittee in veterinar
547–626. Sydney: U
uate Committee in
BLYDE, D. (1990): I
koalas. In *Control*
University of Sydn
Veterinary Science.
BROWN, A. S. (198
PhD thesis, The Ur
Australia.
BUSH, M., GRAVES,
D. E. (1990): Disse
ranging koalas and
Australian Veterina
CLEVA, G. M., ST
(1994): Variation i

als. Such a crisis
appropriate for
application of
hnology, and is
ıs for its general

o understanding
of the species is
he population is
ınd in a reason-
ition. Given that
ıalas still survive
1998) and that
vity (Blanshard,
ecies that could
development of
ılogy. The suc-
and long-term
ı useful manage-
survival of the

ıssisted-breeding
cessful AI in the
ıas not only con-
a better under-
ıtal reproductive
ɔut also should
ıanage and con-
the wild and in
ıt, these studies
rtificial breeding
ınd, with further
ɔgy may also be
he reproductive
ighly threatened
ıern hairy-nosed

ınk Tom Sakurabu,
Koala Sanctuary at
ama, former General
s to facilities and
articular, we thank
ɔon who helped with
and collected Koala
like to acknowledge
Kelly, Bioquest Ltd,
iding the laboratory
ormone assays. We
and National Parks
ɔspital, Moggill, for

allowing access to Koala cadavers for studies of reproductive anatomy. The authors are grateful to Dr F. N. Carrick for providing a critical review of the manuscript. The work presented in this paper was supported by research grants from Lone Pine Koala Sanctuary and the University of Queensland.

PRODUCTS MENTIONED IN THE TEXT

Chorulon®: hCG preparation, manufactured by Intervet Australia Pty Ltd, Unit 3/4 Gladstone Road, Castle-Hill, NSW 2154, Australia.
Isoflurane: gaseous anaesthetic, manufactured by Abbott Australasia Pty Ltd, Captain Cook Drive, Kurnell, NSW 2231, Australia.
Modified foley insemination catheter: A1 catheter, manufactured by Cook Industries Australia Pty Ltd, 12 Electronics Street, Eight Mile Plains, QLD 4113, Australia.
Otoscope: Welch Allyn 3·5 V pneumatic otoscope head with specula, Model No. 20200, manufactured by Welch Allyn Pty Ltd, 4341 Slate Street Road PO Box 220, Skaneateles Falls, NY 13153-0220, USA.
Plastibond: liquid epoxy resin, manufactured by Selley's Pty Ltd, 1/43 Sandgate Road, Albion, QLD 4010, Australia.
Tom-cat catheter: Monoject catheter, manufactured by Becton Dickinson Pty Ltd, 4 Research Park Drive, North Ryde, NSW 2113, Australia.
Vacutainer tubes: EDTA tubes for blood collection, manufactured by Becton Dickinson Pty Ltd, 4 Research Park Drive, North Ryde, NSW 2113, Australia.

REFERENCES

ANZECC (1998): *National koala conservation strategy.* Canberra: Environment Australia.

BARTH, A. D. & OKO, R. J. (1986): *Abnormal morphology of bovine spermatozoa.* Iowa: Iowa State University Press.

BIERY, K. (1991): Sex and the single koala. *Zoonooz* **64**(8): 12–13.

BLANSHARD, W. H. (1994): Medicine and husbandry of koalas. In *University of Sydney post graduate committee in veterinary science proceedings* No. 223: 547–626. Sydney: University of Sydney Post Graduate Committee in Veterinary Science.

BLYDE, D. (1990): Immobilization and anaesthesia in koalas. In *Control and therapy* No. 2495. Sydney: University of Sydney Post Graduate Committee in Veterinary Science.

BROWN, A. S. (1987): *Infertility in the female koala.* PhD thesis. The University of Queensland, Brisbane, Australia.

BUSH, M., GRAVES, J. A. M., O'BRIEN, S. J. & WILDT, D. E. (1990): Dissociative anaesthesia in male free-ranging koalas and selected marsupials in captivity. *Australian Veterinary Journal* **67:** 449–451.

CLEVA, G. M., STONE, G. M. & DICKENS, R. K. (1994): Variation in reproductive parameters in the captive male koala *(Phascolarctos cinereus). Reproduction, Fertility and Development* **6:** 713–719.

HAFEZ, E. S. E. (1993): Assisted reproductive technology: ovulation manipulation, *in vitro* fertilization/embryo transfer. In *Reproduction in farm animals* (6th edn): 461–502. Hafez, E. S. E. (Ed.). Philadelphia: Lea and Febiger.

HANDASYDE, K. A., MCDONALD, I. R., THAN, K. A., MICHAELIDES, J. & MARTIN, R. W. (1990): Reproductive hormones and reproduction in the koala. In *Biology of the koala:* 203–210. Lee, A. K., Handasyde, K. A. & Sanson, G. D. (Eds). Chipping Norton, New South Wales: Surrey Beatty & Sons Pty Ltd.

HILTON-TAYLOR, C. (2000): *2000 IUCN red list of threatened species.* Gland and Cambridge: IUCN.

HOLT, W. V. (1994): Reproductive technologies. In *Creative conservation: interactive management of wild and captive animals:* 144–166. Olney, P. J. S., Mace, G. M. & Feistner, A. T. C. (Eds). London: Chapman & Hall.

HOULDEN, B. A., ENGLAND, P. & SHERWIN, W. B. (1996): Paternity exclusion in koalas using hypervariable microsatellites. *Journal of Heredity* **87:** 149–152.

JOHNSTON, S. D. (1994): *The reproductive biology of the Queensland koala.* Honours thesis, Zoology Department, The University of Queensland, Brisbane, Australia.

JOHNSTON, S. D. (1999): *Studies towards the development of an artificial insemination program in the koala* ***(Phascolarctos cinereus).*** PhD thesis. School of Veterinary Science and Animal Production. The University of Queensland, Brisbane, Australia.

JOHNSTON, S. D., MCGOWAN, M. R., CARRICK, F. N. & CAMERON, R. D. A. (1992): Towards the development of an artificial insemination program for koalas. In *Proceedings of the 12th international congress of animal reproduction, The Hague, Netherlands, 23–27 August 1992:* 1954–1957. Urbana-Champaign, IL: Animal Reproduction Congress.

JOHNSTON, S. D., MCGOWAN, M. R., CARRICK, F. N. & TRIBE, A. (1993): Preliminary investigations into the feasibility of freezing koala *(Phascolarctos cinereus)* semen. *Australian Veterinary Journal* **70:** 424–425 (correction published in *Australian Veterinary Journal* **72:** 473).

JOHNSTON, S. D., MCGOWAN, M. R., CARRICK, F. N., CAMERON, R. D. A. & TRIBE, A. (1994): Seminal characteristics and spermatozoal morphology of captive Queensland koalas *(Phascolarctos cinereus adustus). Theriogenology* **42:** 501–511.

JOHNSTON, S. D., O'CALLAGHAN, P., MCGOWAN, M. R. & PHILLIPS, N. J. (1997a): Characteristics of koala *(Phascolarctos cinereus adustus)* semen collected by artificial vagina. *Journal of Reproduction and Fertility* **109:** 319–323.

JOHNSTON, S. D., MCGOWAN, M. R., O'CALLAGHAN, P., COX, R. & NICOLSON, V. (1997b): Evidence for reflex ovulation in the koala. In *Proceedings of the*

28th annual conference of the Australian society for reproductive biology, Canberra, 29 September–1 October 1997: 33. Canberra: Vertebrate Biocontrol Cooperative Research Centre (CRC).

JOHNSTON, S. D., O'BOYLE, D., FROST, A. J., MCGOWAN, M. R., TRIBE, A. & HIGGINS, D. (1998): Antibiotics for the preservation of koala *(Phascolarctos cinereus)* semen. *Australian Veterinary Journal* **76:** 335–338.

JOHNSTON, S. D., MCGOWAN, M. R., PHILLIPS, N. J. & O'CALLAGHAN, P. (2000a): Optimal physicochemical conditions for the manipulation and short-term preservation of koala *(Phascolarctos cinereus)* spermatozoa. *Journal of Reproduction and Fertility* **118:** 273–281.

JOHNSTON, S. D., MCGOWAN, M. R., O'CALLAGHAN, P., COX, R. & NICOLSON, V. (2000b): Studies of the oestrous cycle, oestrus and pregnancy in the koala *(Phascolarctos cinereus). Journal of Reproduction and Fertility* **120:** 49–57.

JOHNSTON, S. D., MCGOWAN, M. R., O'CALLAGHAN, P., COX, R. & NICOLSON, V. (2000c): Natural and artificial methods for inducing the luteal phase in the koala *(Phascolarctos cinereus). Journal of Reproduction and Fertility* **120:** 59–64.

MARTIN, R. & HANDASYDE, K. (1990): Population dynamics of the koala *(Phascolarctos cinereus)* in southeastern Australia. In *Biology of the koala:* 75–83. Lee, A. K., Handasyde, K. A. & Sanson, G. D. (Eds). Chipping Norton, New South Wales: Surrey Beatty & Sons Pty Ltd.

MATE, K. E., MOLINIA, F. C. & RODGER, J. C. (1998): Manipulation of the fertility of marsupials for conservation of endangered species and control of over-abundant populations. *Animal Reproduction Science* **53:** 65–76.

MCGOWAN, M. R., JOHNSTON, S. D., TRIBE, A., VAN HENSBROEK, L. & CARRICK, F. N. (1995): The use of isoflurane to anaesthetise koalas *(Phascolarctos cinereus). Australian Veterinary Journal* **72:** 472 473.

MOLINIA, F. C., GIBSON, R. J., BROWN, A. M., GLAZIER, A. M. & RODGER, J. C. (1998): Successful fertilization after superovulation and laparoscopic intrauterine insemination of the brushtail possum, *Trichosurus vulpecula,* and tammar wallaby, *Macropus eugenii. Journal of Reproduction and Fertility* **113:** 9–17.

MOLINIA, F. C., NICKEL, M. K., GLAZIER, A. M. & RODGER, J. C. (1997): Intrauterine and intravaginal artificial insemination of PMSG/LH treated brushtail possums. In *Proceedings of the 28th annual conference of the Australian society for reproductive biology, Canberra, 29 September–1 October 1997:* 91. Canberra: Vertebrate Biocontrol Cooperative Research Centre (CRC).

NICKEL, M. K., MOLINIA, F. C., LIN, M. & RODGER, J. C. (1997): Sperm transport in the female reproductive tract of the brushtail possum, *Trichosurus vulpecula.* In *Proceedings of the 28th annual conference of the Australian society for reproductive biology, Canberra, 29 September–1 October 1997:* 68. Canberra: Vertebrate Biocontrol Cooperative Research Centre (CRC).

O'CALLAGHAN, P. (1996): Growth and mortality of koala pouch and back young. In *Proceedings of the Australian koala foundation annual conference, Gold Coast, Australia, 19–21 August:* 101–109. Brisbane: Australian Koala Foundation.

POSSINGHAM, H., BARTON, M., BOXALL, M., DUNSTAN, J., GIBBS, J., GREIG, J., INNS, B., MUNDAY, B., PATON, D., VICKERY, F. & ST JOHN, B. (1996): *Koala management task force: final report.* Roseworthy, South Australia: Department of Environmental Science and Management, The University of Adelaide.

RODGER, J. C. (1990): Prospects for the artificial manipulation of marsupial reproduction and its application in research and conservation. *Australian Journal of Zoology* **37:** 249–258.

SMITH, M. (1980): Behaviour of the koala, *Phascolarctos cinereus* (Goldfuss), in captivity **V.** Sexual behaviour. *Australian Wildlife Research* **7:** 41–51.

WILDT, D. E., SCHIEWE, M. C., SCHMIDT, P. M., GOODROWE, K. L., HOWARD, J. G., PHILLIPS, L. G., O'BRIEN, S. J. & BUSH, M. (1986): Developing animal model systems for embryo technologies in rare and endangered wildlife. *Theriogeneology* **25:** 335–351.

WILDT, D. E., BUSH, M., O'BRIEN, S. J., MURRAY, N. D., TAYLOR, A. & MARSHALL-GRAVES, J. A. (1991): Semen characteristics in free-living koalas *(Phascolarctos cinereus). Journal of Reproduction and Fertility* **92:** 99–107.

WILDT, D. E., SEAL, U. S. & RALL, W. F. (1993): Genetic resource banks and reproductive technology for wildlife conservation. *Nato ASI (Advanced Science Institutes) Series. Series A Life Sciences* **248:** 159–173.

WILDT, D. E., RALL, W. F., CRITSER, J. K., MONFORT, S. L. & SEAL, U. S. (1997): Genome resource banks: living collections for biodiversity conservation. *Bioscience* **47:** 689–698.

Manuscript submitted 8 November 1999; accepted 30 August 2000

Int. Zoo Yb. (2003)

Husbandr

Cercartetus nan

at Healesv

J. A. MURPH

Healesville San

In 1998 6.4 Easte
nanus were placed
enclosure at Heale
two ♀♀ each had li
third ♀ had a sing
conditions which r
1998 and 1999 are
husbandry and re
writing, and to the
first published re
captivity.

Key-words: captive
Eastern pygmy pos

The Eastern p
nanus is a sn
arboreal possur
of habitats i
including coast
and forest (T
1995). The life s
is at least 3 yea
♂♂ (Ward, 199
animal lived for
generalist omn
nectar, pollen,
(Turner, 1984;
et al., 1987;
availability is
timing and d
season (Turner,
Australia breed
November and
year round in
Banksia sp fl
1990a). Males
maturity betwe
age, depending
season of birth
usually produc