REPRODUCTIVE ENDOCRINOLOGY AND ASSISTED REPRODUCTIVE TECHNOLOGIES IN WILD FELIDS

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ABSTRACT

Reproductive endocrinology has advanced a lot beyonds the established hormones dynamics and its application is reaching novel areas. This review article gives insights on reproductive endocrine profiling, artificial insemination (AI), in vitro fertilization (IVF) and embryo transfer in wild felid through exogenous hormonal application. Wild felids are seclusive creatures and they express slight to marked variations in reproductive mechanisms; we lack to clearly define normative data of onset of puberty to reproductive senescence and the understanding of fundamental reproductive physiology. This is crucial in many aspects of conservation in wild and captive breeding. Various researches in bits and pieces revealed that species difference with different estrus period, its length, hormone level were the keys for the failure of AI, IVF and embryo transfer in wild felids. This paper also summarizes the estrogen and progesterone monitoring in female and androgen (testosterone) in male, semen analysis and cryopreservation of sperm and embryo. Reviews of published work reports about 10 offspring born from artificial insemination and 39 offspring born from embryo transfer which are included in this article.

Keywords: *artificial insemination, embryo transfer, in vitro fertilization, wild felids*

INTRODUCTION

There are 38 known wild felids of which 5 (13.2%) are endangered and 13 (34.2%) vulnerable species globally (IUCN, 2020). The major causes of felid population decline in the wild are habitat loss and fragmentation, scarcity of preys and competition with other carnivores, human-animal conflict and poaching. With some exception, most felids do not reproduce well in captivity: relatively success is higher in the cheetah and ocelot but some results revealed that few pregnancies happened in clouded leopard, fishing cat and tiger with artificial insemination (Brown, 2011). It is difficult to establish breeding pairs in captivity because of over aggressive behaviour of male; therefore, assisted reproductive technologies may be desired option to achieve pregnancy.

Factors accounted for successful insemination are likely to be: a) insemination performed at natural estrus without exogenous hormonal treatment, b) minimal manipulation with only one anaesthesia, c) non-invasive transcervical and transuterine insemination, d) high-quality semen, collected through urinary catheter, e) natural mating conditions were mimicked by placing the female in natural mating position (Lueders et al., 2014). In vitro derived embryos are capable of development into different stages and finally production of a new individual after embryo transfer (Dresser et al., 1988; Goeritz et al., 2012; Pope et al., 2006, 2012). Collection of oocytes and insemination in vitro in culture medium to development into the mature fertilized oocytes can be performed in wild cats with exogenous hormonal treatment but differ in success with great (Donoghue et al., 1990; Gómez et al., 2003; Herrick, Campbell, et al., 2010; Thuwanut et al., 2011).

This review highlights the works done in artificial insemination, in-vitro embryo production and embryo transfer in wild felids through exogenous hormone administration and

the possible strategies for conservation of large felids in Nepal through assisted reproductive technologies.

MATERIALS AND METHODS

In identifying the sources for the review, multiple databases were used continuing initially with google scholar and PubMed. Secondary data for necessary information were gleaned through journal articles, book section, conference proceeding, case study, magazine articles, newspaper articles, published reports, technical bulletins and post-graduate thesis. Majority of references cited were within the last 20 years. A few researches were included from late 20th century to establish fundamental concepts that continue to this day.

RESULTS AND DISCUSSIONS

Endocrine monitoring in female wild felids

In wild felids, hormonal evaluation is conducted in feces for determination of conjugated and unconjugated forms of different hormones. In leopard cat, baseline concentrations of fecal progesterone and estrogen were 3.8±1.5 and 0.9±0.2µg/g, respectively. Estrogen metabolite level in feces increased by 6-10 fold (approx. 6-10 µg/g) the baseline before and around copulation and also increased after mid pregnancy whereas fecal progesterone metabolite increased after copulation and increased by 5-15 fold than baseline during pregnancy (approx. 20-60µg/g) (Adachia et al., 2010). Overall mean baseline and peak (in estrus) concentrations of fecal estrogen metabolites were 1307.1±32.8 ng/g and 2962.8±166.3 ng/g. respectively in black footed cat and the lower values were observed in sand cat i.e. baseline concentration 493.9±15.7 ng/g and peak (estrus) concentration 1669.9±83.5 ng/g (Herrick, Bond, et al., 2010). They also revealed that overall mean baseline and peak (in pregnancy) concentrations of fecal progesterone metabolites were 3.6±0.1µg/g and 30.0±1.0 µg/g, respectively in black footed cat and 1.5±0.1 µg/g and 9.4±0.5 µg/g, respectively in sand cat. In contrast, Herrick et al., (2010) also revealed that, after gonadotropin treatment (eCG/hCG) for laparoscopic oocyte collection, the overall mean progesterone concentration peaked to 37.0±3.5 µg/g in black footed cat and 12.3±2.0 µg/g in sand cat. In domestic cat, the levels of mean and peak estradiol in follicular phase in induced ovulator were 85.6±6.2 and109.1±10.8 ng/g feces and mean and peak estradiol in follicular phase and mean and peak progesterone in luteal phase in spontaneous ovulator were 97.8±8.4, 118.4 ± 9.5 ng/g feces and 24.4 ± 1.7, 50.2 ± 4.6 µg/g feces, respectively (Pelican et al., 2008).

In adult African Lions (*Panthera leo*), the overall mean and peak level of fecal estrogen and progesterone were 0.29±0.02, 0.82±0.08, 3.32±0.43 and 11.33±1.25 µg/g respectively (Putman et al., 2015). Fecal basal and peak level of estrogen metabolites in pregnant leopard cat, cheetah, clouded leopard, were observed as 313.7 and 9085 ng/g, 70.6±15.9 and 289±24 ng/g, 33.2±12.6 and 170±36 ng/g, respectively whereas in case of pseudopregnancy fecal basal and peak level of estrogen metabolites in cheetah, clouded leopard, snow leopard were 65.8±5.9 and 527±136 ng/g, 37.5±15.4 and 185±37 ng/g, 225.1±3 5.1 and 1969±61 ng/g, respectively (Brown et al., 1994). They also revealed that the mean concentrations of fecal progesterone level in pregnant leopard cat, cheetah, and clouded leopard were 872.2, 157±46, 99±31, 21±4 ng/g and in pesudopregnant cheetah, clouded leopard and snow leopard were 243±96, 119±31 and 21±4 ng/g, respectively.

Baseline prostaglandin $F_{2\alpha}$ metabolites (PGFM) in feces of sand cat, Asian leopard cat, cheetah, Iberian lynx, ocelot, Sumatran tiger and black panther were 0.04±0.02, 0.06±0.03,

0.12±0.02, 0.04±0.01, 0.52±0.16, 0.06±0.04 and0.04±0.00 µg/g, respectively and the maximum level were 6.5 µg/g, 21.0 µg/g, 1.44 µg/g, 3.63 µg/g, 8.2 µg/g, 2.0 µg/g and 1.38 µg/g, respectively (Dehnhard et al., 2012).

Endocrine monitoring in wild male felids

Overall mean concentrations of fecal testosterone were 3.1 ± 0.1 and 2.3 ± 0.0l µg/g in black footed cat and sand cat, respectively. There was significant (P<0.05) decrease in fecal testosterone concentration during summer (2.9±0.11 µg/gin black footed cat; 2.1 ± 0.1l µg/g in sand cat) compared to the winter (3.3±0.1l µg/g in black footed cat and 2.6±0.1 µg/g in sand cat) (Herrick, Bond, et al., 2010). Average serum testosterone level in bob cat (*Lynx rufus*) was found to be 0.90±0.15 ng/ml and there was no significant effect of season on level of testosterone where in autumn it was 0.79±0.09 ng/ml and in spring 1.33±0.70 ng/ml (Gañán et al., 2009). Higher value of serum testosterone level, 1.5-5.8 ng/ml was observed in leopard cat (*Felis bengalensis*). Production of testosterone in leopard cat was 2-4 fold greater than in normospermic domestic cats and 8-9 fold greater than in teratospermic domestic cats (Jogayle Howard & Wildt, 1990). The mean testosterone level in captive cheetah was 0.44±0.07 to 0.55±0.11 ng/ml and in free ranging cheetah 0.31±0.12 to 0.40±0.13 ng/ml (Wildt et al., 1987).

Semen analysis

The mean number of spermatozoa per ml of ejaculation in captive North American and free ranging East African cheetahs were 25.1±4.4*10^6 and 36.4±12.2*10^6, motile spermatozoa per ejaculate were 26.7±5.8*10^6 and 25.3±9.9*10^6, spermatozoa motility were 70.7±3.5 and 63.1±3.9%, morphologically abnormal spermatozoa were 70.6±3.3 and 75.9±4.4%, respectively (Wildt et al., 1987). Similarly, Donoghue et al., (1992) reported that in cheetahs the ejaculate volume was 1.1±0.2 ml, sperm concentration was 40.6±21.1*10^6/ml, motile spermatozoa per ejaculate were 41.3±22.9*10^6, sperm motility was 74.4±3.6%, and morphologically normal spermatozoa per ejaculate were 28.4±4.9%. Mean ejaculate characteristics of five clouded leopards were; ejaculate volume 1.2±0.1 ml, sperm concentration 43.8±16.7*10^6/ml, sperm motility 72.0±3.4% and morphologically normal spermatozoa per ejaculate 15.4±4.5% (J. Howard et al., 1996). In case of Black-footed cats (BFC) and Sand cats (SC), the mean ejaculate volume was 246.5±11.8 and 200.3±18.0 µl containing sperm concentration of 130.4±23.6*10^6/ml and 209.8±38.3*10^6/ml among which mean sperm motility was found to be 82.5±1.9% and 78.3±1.3%, and the morphologically normal spermatozoa per ejaculate were 46.8±3.0% and 40.4±3.1%, respectively (Herrick, Bond, et al., 2010).

Mean ejaculate volume in the Leopard Cat (*Felis bengalensis*) was 148.3±14.9µl containing sperm concentration 37.0±l5.4*10^6/ml, sperm motility 73.8±2.6% and structurally normal spermatozoa 65.4±2.0% (Jogayle Howard & Wildt, 1990). Semen analysis for mean value of different factors in Jungle Cat (*Felis chaus*) were reported as; volume of ejaculation 69±27.92µl containing 75.13±17.05*10^6/ml of spermatozoa having 77.13±14.15% motility and 73.8±6.09% morphologically normal spermatozoa (Kheirkhah et al., 2017). Mean volume of one ejaculation in African lion (*Panthera leo*) was 422.86±296.07ml containing mean sperm concentration of 1.94±1.61*10^9/ml and the mean percentage of motile sperm was 84.14±20.35% in Ham's F-10 culture medium and 68.94±23.35% in bovine culture medium (Lueders et al., 2012). Gañán et al., (2009) reported that, mean ejaculate volume in

bobcat (*Lynx rufus*) was 362.69±84.06 µl having sperm concentration of 24.40±7.87*10^6/ml and there was a significant effect ($P<0.05$) of season on sperm concentration ; sperm motility was 55.71±5.76% and 14.67±2.07% morphologically normal spermatozoa which was significantly affected ($P<0.05$) by season. Breeding in captivity

Cryopreservation of sperm and embryo

Different researchers used same process of cryopreservation of sperm in liquid nitrogen with different semen extenders. Fresh semen sample was extended separately in TEST yolk buffer containing 4% glycerol in 1:1 ratio containing 25–50 million motile sperms/ml and cooled to 4°C and then transferred to dry ice 5 minutes before storage in liquid nitrogen (Herrick, Campbell, et al., 2010; Pope et al., 2006). Similarly, Gañán et al., (2009) used two cryodiluents: (a)TEST containing 4% glycerol and (b) Biladyl containing 4% glycerol and straw containing cryodiluent from room temperature (20 °C) lowered to 5 °C in 120 minutes and then stored in liquid nitrogen. In a study, sperm was diluted in 1:1 ratio with Tris-glucose-citrate containing 20% (v/v) egg yolk, 8% glycerol and 1% (v/v) Equex STM Paste making final concentration of 50 million motile sperms/ml at room temperature then straw were cooled at 5 °C and finally stored in liquid nitrogen (Thuwanut et al., 2011; Zambelli et al., 2010, 2008). Similarly, according to Swanson et al., (1995), the sperm pellet was firstly diluted with Ham's F10 medium then was extended in cryoprotectant diluent containing 20% egg yolk and 4% glycerol to a concentration of 140-160 million motile spermatozoa per ml, cooled in a refrigerator at 5 °C for 30 minutes and then frozen by pelleting onto dry ice and finally deposited the resulting pellet into liquid nitrogen.

In a study, cryoprervation of embryo in cryoprotectant solution consisted of 1.4M propylene glycol, 0.125M sucrose, 10% dextran70 and 10% FBS in HeTy. On Day 2 (4- to 8-cell), Day 4 (early morulae) and Day 5 (morulae) of in-vitro cryopreservation and embryos were loaded in straw. Embryos were placed in cooling chamber and cooled at 2 °C /min to -6 °C and the after 10 min hold cooling was resumed at 0.3 °C /min to -30 °C, and after a 10 min hold, embryos were plunged into liquid nitrogen (Dresser et al., 1988; Gómez et al., 2003).

Artificial insemination (AI)

Assisted reproduction techniques including artificial insemination (AI) have considerable potential for managing endangered felids and maintaining genetic diversity in small populations. This strategy would be particularly useful for species like the clouded leopard (Neofelis nebulosa) that demonstrate behavioral incompatibility and reduced reproductive performance (Yamada and Durrant, 1989). Artificial insemination in clouded leopard (*Neofelis nebulosa*) using laparoscopic intrauterine process was performed and ovarian activities were monitored in 2 different trials (J. Howard et al., 1996).; in trial 1, evaluation after 29-39 hrs after hCG treatment, only two of nine (22.2%) females had fresh ovulation sites, and one female had mature corpus luteum (CL) and serum progesterone concentration was higher (28.2 ng/ml) only in leopard with mature CL. In trial 2, dose of gonadotropin was lowered (50-100 IU) and interval between hCG (75 IU) to laparoscopy was made longer (>43hrs.) and in four of the five leopards ovulation taken place. One mature CL was seen in a leopard and was capable of forming 8 fresh ovulation sites. Serum progesterone was elevated above baseline (4.5 ng/ml) in one clouded leopard having fresh CL and the highest level (34.2 ng/ml) in leopard having evidence of mature CL. Laparoscopic AI was

performed after 45.4±0.7 hrs of hCG injection and single pregnancy was produced with birth of 2 live offsprings (one male, one female) after 89 days of gestation (J. Howard et al., 1996).

In another study, female tigress was injected 1000 IU eCG followed by 750 IU hCG after 80 hours and laparoscopic artificial insemination was performed after 46 hours of hCG administration with 16.8 million motile spermatozoa. After full term pregnancy (111 days), a single healthy male cub was born (Donoghue et al., 2016). Similar case was reported by Chagas et al., (2000) with same hormone administration and transvaginal artificial insemination after forty-one hours of second hormone administration with fresh semen containing 500 million motile spermatozoa collected by electroejaculation and after full term pregnancy (103-day), the tigress gave birth to three apparently healthy cubs that survived 24–48 hrs.

On the fourth day of normal estrus in Persian leopard (*Panthera pardus saxicolor*), 2000 IU of hCG was administered and 20 hours post-hCG administration, insemination was performed with fresh unextended semen (2ml) with sperm concentration $1.5*10^8$ per ml and exhibited excellent motility collected by electroejaculation and additional 1000 IU hCG was administrated following the first insemination. A second insemination was accomplished 40 hours post-hCG. After full term pregnancy of 96 days, a stillborn male cub was delivered; the cub was in breech presentation and there was the complication of dystocia which was the cause of death of cub (Dresser et al., 1982). In Asiatic Golden Cat (*Catopuma Temmincki*), female was scanned transcutaneously to assess follicle (one preovulatory follicle on each ovary) development and accuracy of timing and then 0.03 mg of Burserelin (GnRH analogue) was administered followed by insemination 2.5 hours later with 88 million sperm concentration and about 70% motility. Parturition occurred on day 84 of gestation and two live cubs were born suggesting that both preovulatory follicles seen had ovulated and fertilized (Lueders et al., 2014). In contrast, Pallas' cats (*Otocolobus manul*) were given 100–300 IU eCG and after 80 hours 75–150 IU hCG was given to induce ovulation and then laparoscopic AI was conducted after 36-38 hrs of hCG administration but successful AI had not achieved (Brown et al., 2002). Similarly, anestrus female ocelots (*Felis pardalis*) were treated with eCG and after 80 hrs hCG, at four gonadotropin dosages and laparoscopic insemination was performed in 10 females 40 hrs after hCG treatment. Only one of the 10 was pregnant with cryopreserved semen and carried full term pregnancy (Swanson et al., 1995).

Invitro fertilization (IVF) and in vitro maturation (IVM)

There was an approximately 10-year interval between the first report of the birth of kittens after embryo transfer (ET) in the cat, until the birth of kittens after IVF/ET and after embryo cryopreservation/ET. Since then, techniques for the in vitro production of cat embryos have developed sufficiently to allow up to one-half of all in vitro embryos to develop into blastocysts in vitro and births of kittens after transfer of embryos derived by a variety of in vitro techniques (Pope et al., 2006). In vitro fertilization of 109 oocytes were performed with controlled cryopreserved semen and 173 oocytes with Equex STM paste containing semen where cleavage occurred in 60 (55.0%) and 86 (49.7%) oocytes, respectively. On day 6, evaluation revealed that 26 (23.9%) morulae and 18 (16.5%) blastocycts were formed from controlled semen and 54 (31.2%) morulae and 23 (13.3%) blastocycts from Equex STM paste semen. Matured blastocysts/cleaved were 29/60 (48.3%) and 42/86 (48.8%) (Zambelli et al., 2010). Similarly, freshly collected BFC and SC spermatozoa were used to inseminate 41 oocytes from Black-footed cats (BFC) and 65 from Sand Cat (SC) among which 23 and 33 were cultured in feline-optimized culture medium (FOCM) while 18 and 32 in Ham F-10

nutrient mixture (HF10) respectively. After 48 hours of insemination cleavage in BFC were 73.7±11.0% in FOMC and 58.8±15.6% in HF10 whereas in SC it was 100.0±0.0% in FOCM and 62.1±20.9% in HF10. In SC, the use of cryopreserved semen did not affect (P>0.05) the proportion of oocytes cleaving at 48 hours (61.5±14.8%) after insemination. 14 oocytes from a single BFC were inseminated in FOCM with cryopreserved BFC spermatozoa and after 20 hours only 4 oocytes had cleaved but no additional cleavage occured after that (Herrick, Campbell, et al., 2010). Gañán et al., (2009) reported that oocytes were incubated with frozen bobcat spermatozoa which was cultured with HEPES-buffered Tyrode's medium and cleavage was evaluated 44 to 48 h after insemination. Percentages of cleavage were 24.4% (31 of 127) for spermatozoa cryopreserved in TEST and 2.9% (1 of 34) for those cryopreserved in Biladyl. Further development was observed and all embryos were reached the morula stage. Out of 26 embryos cultured, 1 was found at the 4-cell stage, 18 had 16 and 30 nuclei, and 7 embryos developed to the ≥32-cell stage.

In another study, a total of 384 oocytes were collected from ovaries of domestic cats and inseminated with cryopreserved ejaculated sperms. Then the cleavage rate was recorded at 24 and 48 h post insemination and mean rates of morula was 17.2±3.6 % and blastocyst was 13.6±4.5%. The percentage of morula stage when domestic cat oocytes were inseminated with cryopreserved flat-headed cat sperm supplemented with GPx (14.5±6.0) and vitamin E analogue (9.3±9.9) was not higher (P>0.05) than in the control samples (8.3±4.5) and did not significantly differ from the domestic cat sperm group. The percentage of blastocysts was 1.1±2.9 with control, 9.3±9.9 with vit-E analogue and 4±4.5 with GPx. In addition, the percentage of blastocyst development derived from flat-headed cat sperm samples was significantly lower than the domestic cat sperm samples (Thuwanut et al., 2011).

In cheetah, 277 oocytes were collected and 214 mature oocytes were inseminated, 56 (26.2%) were fertilized and 37 (17.3%) cleaved to 2-celled stage after 30 hours but the incidence of IVF varied from 0 to 73.3% (P<0.001). Evaluation of embryo after 48 and 72 hours revealed that there were 25 and 19 embryos among which and 7 (28.0%) and 2 (10.5%) were in 4-celled stage, 17 (68.0%) and 7 (36.8%) were in 8-celled stage, 1(4.0%) and 10 (52.6%) were in 16-celled stage respectively. There was positive correlation (P<0.001) between the number of sperm reaching the inner half of zona pellucida and fertilization rates. Likewise, the number of sperm used per insemination had no influence (P>0.05) on subsequent IVF rates (Donoghue et al., 1992).

In another study, a total of 456 oocytes were collected (mean 28.5±3.4 oocytes/female) from tigress and 358 mature oocytes were inseminated. Likewise, in 195 fertilized oocytes, 187 (95.9%) were in 2-celled stage. Of these, 56 cleaved embryos were cultured in Ham's F10 for 72 hours, 14 (25.0%) were in 16-celled stage and 15 were in morulae stage and 466 embryos cultured for 92 hr, 20 (43.5%) were in advanced morulae and 14 (30.4%) in early blastocysts (Donoghue et al., 1990). In a study by Goodrowe et al., (1989) in leopard cat, 47 and 27 oocytes were collected laparoscopically in 80 and 84 hr after hCG administration with mean of 4.7±1.2 and 5.4±1.7 respectively and cocultured with processed, homologous spermatozoa. Of these, total mature oocytes fertilized with two-celled stage were 17.5% and 52.4% in 80 and 84 hr respectively and were significantly affected by time of collection(P<0.005).

Embryo transfer

Harvesting oocytes, in vitro fertilization and embryo transfer (ET) are the assisted reproductive technologies for conservation of endangered felid species. In a study (Pope et al., 2006), multiple laparoscopic oocyte retrievals were done in fishing cats (*Prionailurus viverrinus*), caracals (*Caracal caracal*) and domestic cats after ovarian stimulation with gonadotropins. Total 579 preovulatory oocytes were recovered and 348 embryos were produced in vitro, for an overall cleavage frequency of 60% in fishing cat and 452 preovulatory oocytes were recovered and 297 embryos were generated for a cleavage frequency of 66% in caracal. Cleavage frequency in fishing cat and caracal after in vitro fertilization (IVF) with frozen semen was 71% (221/313) and 64.8% (254/392), with semen stored at 4^{o}C for 24 h was 55% (36/65) and 71.7 (43/60) respectively. In fishing cat, 12 embryos (fertilized with stored spermatozoa) were transferred and one pregnancy (8%) was obtained and one live female kitten was born on day 63 of gestation. Similarly in caracal, among 6 embryos (fertilized with stored spermatozoa) transferred, one pregnancy (16.67%) was obtained and 2 live female kitten were born after day 77 of gestation period while among 9 embryos (fertilized with frozen spermatozoa) 3 pregnancies were seen and three live kittens were born after 80 days of gestation (Pope et al., 2006).

In a study in African Lions (*Panthera leo*), female lion was hormonally influenced (eCG, hCG in group I and pLH, pFSH in group II) in two groups with 2 protocols and transvaginal insemination with extended semen while embryo collection was performed surgically in 3 animals (2 from group I and 1 from group II). Whereas in animals of group I, only two unfertilized oocytes and one two-cell stage embryo were flushed out of the uterus and in animal of group II, three apparent embryos at the late morula stage were recovered and the embryos were cryopreserved by vitrification (Goeritz et al., 2012). Similarly, surgical embryo recovery was performed in *Felis catus* after 11-13 days of pFSH administration and recipient female was synchronized followed by surgical embryo transfer (frozen-thawed) at the anterior tip of horn was performed. All embryos transferred ranged in stage from late morula to expanded blastocyst and 5 of 11 females were pregnant and after full term pregnancy (mean=66.4 days), 15 live healthy kitten and 2 still birth kitten were born (Dresser et al., 1988).

In five black footed cat (*Felis nigripes*), 2 received fresh embryo whereas 3 received cryopreserved embryo and only 2 females with cryopreserved embryo were pregnant for full term and 2 live males and a live female kitten were born without assistance (Pope et al., 2012). They also reported that, in domestic cat after embryo (from in vivo- and in vitro-matured oocytes) transfer, three male and two female kittens were born. However, 4 of the five kittens had to be deliverd by C-section and two kittens died during labor and two were with anatomical abnormalities died shortly after delivery and the other died from respiratory infection/pneumonia at day 15. Similarly, in USA, two laparoscopic embryo transfer procedures were attempted in ocelot (Leopardus pardalis) and both resulted in pregnancy, resulting in birth of one kitten (Swanson, 2001). Subsequently, in Brazil, transfer of three frozen ocelot embryos resulted in birth of one still born Brazilian ocelot kitten (Swanson, 2002). Gómez et al., (2003) reported that, total 15 female were subjected to embryo transfer among which 4 were pregnant. Two of the pregnant females had one fetus, one had two fetuses and another had three fetuses where one female delivered a live healthy kitten and second female, after C-section produced a live male kitten. One recipient that had two fetuses, one of them was reabsorbed at approximately 40 days of gestation, and one kitten was born

but died during parturition with no abnormalities. In the remaining pregnant female, all three fetuses were aborted at about 45 days of gestation. In a female tiger (*Panthera tigris*) after embryo transfer, 3 live cubs were born assisted by caesarian section. One of three cubs died within 1 hour of birth because of respiratory complications (Donoghue et al., 1990).

CONCLUSION

Identifying the type of ovulation (induced vs. spontaneous) in felids and the effect of season on reproduction for each species is important because these two characteristics impact both natural and assisted breeding efforts. Lower number of follicle stimulation and oocytes collection, fertilization in vitro, large number of degradation of oocytes and low pregnancy rates following AI in most wild felids may be partially attributed by less known hormonal stimulation protocols. Species differences may be significant and can easily lead to ovarian hypo or hyperstimulation. It has been proven that in wild felids, follicular development can be induced by equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG) as well as with porcine LH and porcine FSH. Further research should be done to know the proper dose of hormone in different species. Some of the research works were successful in the development and fertilization of oocytes in vitro and capable of advancing to morulae and blastocysts in culture and live born offspring after embryo transfer. Nepal has successfully doubled the tiger count and from conservation point of view in large wild felids artificial insemination, in vitro fertilization/maturation and embryo transfer plays a key role.

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