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A Suggested Birth Protocol for Bottlenose Dolphins (*Tursiops truncatus*) – Updated 2015, Zoo Nuremberg

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Introduction

Reproduction is a very important aspect for zoos and essential for their animals. With the new dolphin lagoon, which opened in 2011, Zoo Nuremberg wanted to emphasize this issue. The lagoon consists of nine different pools (three indoor and five outdoor). The maternity pool (12 m in diameter and 3.5 m deep) is an indoor pool equipped with a lifting floor to allow for better access to newborn calves and their mothers. This protocol includes a lot of data and knowledge from other marine mammal facilities and from colleagues with experience in the birth and subsequent care of dolphin calves and their mothers. It was established to offer a standard procedure during pregnancy and postpartum, and it may be beneficial to other facilities as a management tool during the reproduction period of their bottlenose dolphins (*Tursiops truncatus*).

Suggested Protocol and Procedures

In preparation for dolphin births at Zoo Nuremberg, we searched the literature and consulted with various institutions that keep dolphins to develop a protocol for the prepartum, partum, and postpartum periods for bottlenose dolphin reproduction. Results from the Third Dolphin Neonatal and Reproduction Workshop in Indianapolis and the First Neonatal Workshop in Paris, both in 2005, were also considered. Our aim was to gain as much information as possible and produce a protocol that could also be followed by other institutions. Comments are added to the different sections in the following protocol to indicate how applicable a procedure has been and whether adaptations were, or might be, needed as each facility and every animal are different.

This protocol is divided into the following sections: (1) preparation, (2) female prepartum (3) female intrapartum, (4) female postpartum, and (5) calf postpartum. Each section is outlined below with suggestions on procedures that have proven successful at Zoo Nuremberg:

1. **Preparation** – Tasks to be carried out during pregnancy to prepare the mother as well as the human teams and equipment in advance of parturition:

    a. Select the observation team and establish a time schedule—Two persons are scheduled for each night shift, and the most experienced trainers are chosen for the predicted time of birth and the days thereafter.

    b. Procure hygienic protective clothing for team members working with the calf.

    c. Install disinfection stations for hands and shoes at every entrance to the mother–calf area.

    d. Make a preliminary decision on the water temperature for the calf—At Zoo Nuremberg, which has indoor and outdoor pools with seasonal changes in temperatures, it was decided that the water temperature for the newborn calf should not be below 16°C.

    e. Determine the water quality level—Bacteriological and mycological tests should be done weekly on a routine basis.

    f. The following equipment should be ready:

        i. A scale for weighing the calf

        ii. A floating veterinary raft, very useful for keeping all material

needed for intervention of the calf safe and dry (see Figure 1)

iii. A blood glucose meter pretested on dolphin blood

iv. An instant blood analysis device

v. A mammary (breast) pump developed especially for dolphins (see Figure 2)

vi. Gastric tubes and syringes for tube feeding—The tubes should be 8 to 10 mm in diameter and 75 cm in length. The volume of the syringes can be between 70 and 100 ml.

vii. A bottle for feeding the calf (in case hand rearing is required). Various options can be used—for example, a bottle for lambs with a special nipple.

viii. Ingredients and recipe for calf formula

ix. Cameras—Two to three underwater cameras should be placed at different levels and angles, with one directly at the underwater window (if available) so as to have the best overview. Most important is that observers have 100% visibility of the mother and neonate. This is necessary for accurate documentation of nursing performance.

x. The air conditioning in the indoor pools should be checked; the air stream should not be directed at the water surface.

xi. Hydrophones can be installed in the maternity pool for cooperating with scientists.

xii. Press release—If prepared beforehand (including all eventualities), this saves time when the calf is born.

xiii. Preliminary practice—Dry runs are very useful. The exact position of the teams, time frames, and means of communication can be tested on inflatable dolphins.

Formula for Newborn Calves in Nuremberg—The formula should be prepared in the morning. The first portion is used immediately, and the rest is frozen in 23 portions. Each portion is then defrosted 10 min before feeding and then given at 37°C. The Zoo Nuremberg formula is as follows:

- 1,125 g Herring fillet with guts
- 97.5 g Zoologic 30/55 (Multimilk®, PetAg)
- 75 ml Salmon oil
- 3 tablets Lactobacillus (Paidoflor®, Ardeypharm)
- ¾ capsule Taurin 500
- 3 Sea Tabs for birds, turtles, fish, and smaller sharks® (Pacific Research Laboratories)

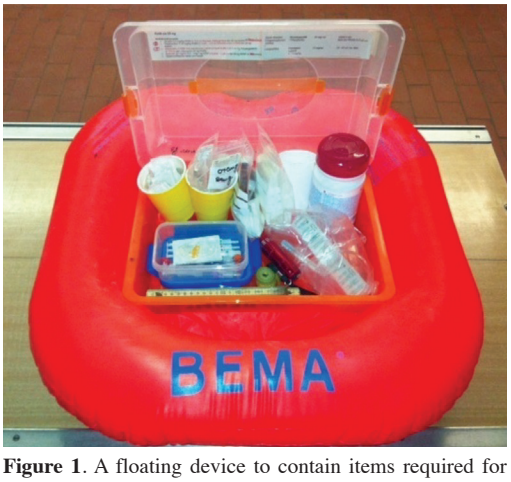

Figure 1. A floating device to contain items required for intervention and care

Figure 2. Mammary (breast) pump developed by Walb for dolphin application

- 3 tablets Calciumcitrat – Calciumglycerophosphat (Calcipot®, Meda Pharma)
- 10 g Lecithin pure granulate
- 1,650 ml Water
- Dimeticon (Sab Simplex®, Pfizer)—A tensioactive that can be added to the formula

The feed amount and feeding intervals depend on the weight and age of the calf as well as the estimated energy requirement of 150 to 200 kcal/kg/d (Townsend & Gage, 2000). Initially, feedings of 20 to 30 ml every 30 min have worked well. These can be increased slowly to 50 ml per feeding. It is advisable to dilute the formula with water by 50% for the first feeding, by 40% for the next feeding, then 30%, and so on, until the full formula is fed. After every feeding, the tubes and/or bottles should be cleaned and disinfected.

Comment: Having this "checklist" ready and providing everything well in advance allows the focus to be on the animals. A good observation team is essential. Preparation of all technical equipment can be very time-consuming. Installing it ahead of time allows for preliminary testing. At the EAAM symposium in Genoa, a feeding technique called "Spiderman" was presented by Oceanografic (Issenjou et al., 2017).

2. Female Prepartum – As fish eaters, dolphins under human care need vitamin supplements. Various marine mammal products are available on the market. To ensure an adequate supplementation during pregnancy, individually balanced vitamin regimens might be considered. At Zoo Nuremberg, the bottlenose dolphins are supplemented individually with single vitamins. During pregnancy, supplementation was changed based on specific research (Gimmel et al., 2016) and blood results of the female. After consultation with Professor Liesegang of the Department of Animal Nutrition at the University of Zurich, we increased the vitamin B1 and added folic acid to make the following formula: vitamin B1 200 mg TID, Folsan® (Teofarma) 5 mg BID. The amount fed does not need to be increased prepartum. Weight management of the mother is essential, along with growth management of the fetus (monitored by ultrasound). By controlling the weight gain of the mother (especially in the third trimester), we are able to control the size of the fetus to avoid an oversized fetus at parturition, which can result in stillbirth.

The following points should be considered in the prepartum phase:

a. Blood samples should be taken on a regular basis.

b. The rectal temperature can be measured to better predict birth (birth normally occurs 24 h after the temperature has dropped by 1°C). To accustom the animals and trainers to this procedure, the following schedule is suggested:

   i. 3 mo before birth: once a week

   ii. 2 mo before birth: once a day

   iii. 2 wks before birth: twice a day

c. Measuring the distance between the mammary glands also gives an indication of milk production and the approaching birth:

   i. 3 mo before the birth: once a week

   ii. 2 mo before the birth: once a day

   iii. 2 wks before the birth: twice a day

d. Training with the mammary (breast) pump (in case it becomes necessary)—The female needs to learn the correct body position and become accustomed to the feel of the suction created by the breast pump.

e. Ultrasonography can be used as follows to check the vitality of the fetus and predict the time of birth using the prediction program developed by Lacave et al. (2004):

   i. In the first half of pregnancy: once a month

   ii. Beginning at Month 7: twice a month

   iii. Beginning at Month 10: weekly

   iv. Beginning at Month 11: twice a week

v. Beginning 3 wks before the predicted date: 3 times a week

vi. In the last week before the predicted date: daily

vii. The following parameters are determined in every ultrasound session: the position of the calf, measurements of the skull and thorax, and the heart rate for at least 1 min (Saviano, 2013).

f. The animals should be trained on the lifting floor in advance. It is important for prospective mothers to become accustomed to a regular presence in the medical pool. During this time, they should receive tactile handling and other forms of positive reinforcement. This conditioning enables handling intervention with the neonate without a negative reaction from the mother:

i. At various levels, including a depth of 50 cm (approximate depth for handling the neonate)

ii. Separation of mother and calf should be simulated with all predicted procedures and tools in place

g. Parturition phase—It should be decided whether to separate the female for this event or to keep her together with others. In some institutions, inexperienced females are present in the birthing pool, especially if the birthing mother is experienced and has had previous successful births. It is important for young females to experience normal births so that when it becomes their turn, they will be familiar with the process, especially what occurs in the first several minutes. At the same time, staff must be prepared to remove any dolphin that persists in interfering with mother and neonate.

h. Separation training—For all eventualities, the female should become used to being separated from the group. (This can be done for short periods at first and then prolonged gradually.)

i. Lighting regimen in the maternal pool—For observation purposes, the lights need to be switched on during the dark hours, and the female should be made accustomed to this (a few weeks before birth).

Comments: For an example from Zoo Nuremberg, the feed amount was not increased based on previous experience, but also on recommendation from other facilities. The vitamin regime was adapted on the basis of the work of Gimmel et al. (2016). The rectal temperature was 36.4°C (97.5°F) 3 mo prior to birth, dropping to 35.9°C (96.7°F) 2 mo prior to parturition, before returning to 36.4°C (97.5 °F). The temperature dropped again to 35.8°C (96.4°F) 1 mo prior to parturition, and then rose again and dropped to 35.7°C (96.2°F) 2 d preparturition and to 35.6°C (96.0°F) 1 d later. Changes in intramammary distance gave us the following results: 3 mo prior to birth, the distance was 3.3 cm; a month later, it had increased to 3.5 cm, where it stayed until birth. Thus, this measure was not very conclusive. This prognostic tool did not prove to be of great value in our case. Having developed a new breast pump beforehand gave us a chance to train the animal with it.

Ultrasonography is an extremely important aspect—not only for following pregnancy, but also for predicting the date of birth. Ultrasound control is the state-of-the-art tool for reliably confirming a pregnancy (Williamson et al., 1990; Brook, 1997; Stone et al., 1999; Lacave et al., 2004). Ideally, females should be checked on a weekly basis to identify the development of any follicles on their ovaries and to follow their subsequent cycle (Brook, 2000, 2001). Ultrasonographic measurements provide the best predictions during the first third of gestation. Later in gestation, there is more variability, particularly in thoracic diameter, depending on the fetus's own genetic background. We used ultrasonographic measurements of the fetus to predict birth through bi-parietal and thoracic diameters (Lacave et al., 2004) and were able to determine the date of birth precisely. In our case, the predicted day of birth using both measurements differed by 6 d from the real event, but it also worked using only the measurement of the head (6 d) and the thorax (4 d) and has proved to be a very valuable tool. At Zoo Nuremberg, the female was separated, although all of our dolphins were in the adjacent pools and could watch the female and interact with her acoustically through a separation mesh, which they actually did.

3. **Female Intrapartum** – Parturition is a very sensitive and sometimes also surprising moment and, therefore, it is important to be well prepared. Some decisions need to be taken in advance, and everything should be ready in case of emergencies:

    a. Maternity pool—In our institution, the female stays in the pool with the lifting floor.

    b. The water depth is not changed.

    c. Observations—Two experienced persons are present to record signs of labour, the behaviour of the female, and vaginal discharges.

    d. Ideally, parturition should not last longer than 2 h. While some females are very slow in the first portion of parturition, this is not necessarily a problem as long as the fetal flukes, or rostrum, are fully visible. Once the contractions become regular in frequency and of full strength, fetal protrusion progresses. It is when the umbilical cord enters the birth canal that the progression time becomes critical.

    e. Distocia or worst case scenario—There may be different situations. For all, a protocol should be available. Examples are listed below:

Loss of Blood/Tissues – With No Sign of Delivery
Direct ultrasonography (voluntary or through the lifting floor) to determine the viability of the calf.

Delayed Partum – Dystocia
When labour continues for too long without progression, administer Oxytocin, 30 IU IM, once per hour.

Placental Retention or Incomplete Placenta
Ultrasonographic control of the uterus (and identification of retained material): Oxytocin, 30 IU IM, PGF2alpha IM (0.16 mg/kg), and intra-uterine washes

Fetal Death
Early diagnosis is of utmost importance. As soon as one notices an absence of heartbeat and/or a loss of homogeneity in the amniotic fluid, it is mandatory to carry out ultrasonographic control daily and blood sampling every other day to identify the evolution and/or the possible development of infection. Early in gestation, the female generally aborts on her own. Later, she will need help. One should not wait to intervene in this situation. Manual extraction may become necessary (Lacave, 1991; Gili et al., 2005). During delivery, if the cervix is open, Oxytocin 30 IU IM is given once per hour.

Provoked Abortion
PGF2alpha IM (0.16 mg/kg), Dinoprostone (PGE2) cervical gel (e.g., Prepedil-Gel®, Pfizer), Prostin E2 (0.5 mg/h), and corticoids can be used. The female should be put under strong antibiotic protection (Amikacin) and strong pain killers (Tramadol, 1mg/kg). If manual extraction becomes necessary, anaesthesia may be considered.

Foetotomy Material
One has to be prepared for this eventuality. It is advisable to have all the material and instruments on site or located ahead of time so that they are available if needed. Establishing contact with practitioners experienced in foetotomy is strongly recommended. Anaesthesia may be considered (A. Sogorb, pers. comm., 2005).

Distocia Protocol at Zoo Nuremberg

- In case of loss of blood or other tissues, without a sign of ongoing birth, ultrasonography should be used to determine if the calf is alive.
- Oxytocin—30 IU (dependant on the degree of cervical opening)
- Control, if cervix opens
- If not (and the calf is dead)—Dinoproston (Prepedil-Gel®, Pfizer) applied locally
- If the cervix opens, manual extraction (possibly under water if parts of the calf can be seen)
- See also the CRC handbook, *Marine Mammal Medicine*, Chapter 30 (Walsh & Gearhart, 2010).

Comments: In this critical situation, it is important that the observers are experienced. If parturition takes longer than expected, they have to be able to decide on the next steps. In case of distocia, the team needs to be ready to intervene.

4. **Female Postpartum** – The first days after parturition are the most sensitive, and it may be necessary to make adaptations when handling the female. Medical aspects need to be considered, and the behaviour of the mother is a crucial element in this phase. If needed, antibiotic treatment, Amoxicillin/Clavulanic acid p.o., is recommended for the first 5 to 7 d postpartum (Lacave, 1994).

    a. Feed—The amount is to be increased on demand on a 24-h feeding regime:

        i. Typically, postpartum feed consumption increases by 50% or more over typical baseline maintenance consumption.

        ii. Feed intake and milk output peak at about the same time (Days 7 to 10) (Sweeney et al., 2010).

    b. A milk sample should be obtained, analyzed, and, if possible, stored.

    c. Incomplete placenta delivery—In this case, oxytocin should be given by injection. The placenta should be weighed, photographed, and submitted to a laboratory check with anatomical and pathological dissection, including bacteriology.

    d. In case of anxiety or aggression,

        i. The water level can be changed.

        ii. Interactions with the trainers might help.

        iii. A different feeding regime can be applied.

        iv. Diazepam p.o. can be considered if the mother is agitated and cannot be calmed by training or a modified management.

Comments: At Zoo Nuremberg, the amount of feed was increased to a maximum of 18 kg on Day 17. It then stabilized at around 14 kg/d. Milk samples were obtained easily with the newly developed breast pump. The placenta was retrieved and sent for dissection.

Results: Macroscopy and histology revealed no abnormalities, and the PCR was negative for Coxiella and Chlamydiaceae species.

5. **Calf Postpartum** – The calf should be observed very closely for the first 4 wks of life. Development can be followed using various parameters, thereby detecting potential changes. Interventions on the calf should be planned in advance, and the important steps defined. In case the mother does not accept the calf, and no foster mother is available, a hand-rearing protocol should be available.

    a. Observation—24-h observation for the first month to include the following parameters:

        i. Breathing frequency—The observation period should last 5 min because there can be big differences during nursing and playtime. Thereafter, the results are averaged. Neonates normally breathe 12 to 25 times in 5 min (Sweeney et al., 2010).

        ii. Nursing frequency and time:

            (1) Nursing normally starts after 3 to 8 h (a few mothers wait until the placenta has been delivered).

            (2) After 12 h, the situation of the calf becomes critical if it has not nursed.

            (3) After 24 h at the latest, if nursing has not been observed, an intervention is necessary.

            (4) Three to five nursing events are normal, followed by a 15- to 20-min break.

            (5) The total duration is important and is counted in seconds per hour. A decrease in this value over time is a sign of successful nursing.

                Example: Day 2, 50 to 70 s/h; Day 8, 8 to 35 s/h (Sweeney et al., 2010)

            (6) The nursing frequency should be observed for at least 2 wks (Sweeney et al., 2010).

iii. Urinary and fecal frequency:

(1) Fecal excretions should be observed by Day 3 and may be seen several times per hour. Feces should be of a thick liquid consistency, light grey in color, and should not float or exhibit gas bubbles.

(2) CAVE—Constipation can be a problem (Sweeney et al., 2010).

iv. Daily photographs render it possible to see the changes in the skin and weight as well as eventual lesions.

Comments: At Zoo Nuremberg, the average breathing rate in the first days was 14.6 (±2) breaths in 5 min. We recorded the number of breaths the calf took with the mother and how many it took on its own. After a few days, the breathing intervals of 14 breaths in 5 min (±6) varied greatly, depending on whether the calf was playing or resting. Nursing started 9 h after the birth. We measured the frequency and the duration in seconds and then calculated the seconds per hour and then minutes per day. We started with a maximum of 342 s/h on Day 1, and reached values of 30 to 60 s/h on Day 14. In minutes per day, the nursing events reached a maximum of 58 min/d on Day 2, then decreased to 19 (±2) min/d on Day 10, 12 (±2) min/d on Day 20, and 10 (±2) min/d on Day 30, where they remained until Day 60. We made note of every micturition and defecation during the 24-h observation periods. Daily photographs were very helpful in observing calf development (weight and skin colour) and evaluating small skin lesions on a daily basis.

b. Intervention on the calf

Capture: Intervals should be fixed prepartum and altered in case of abnormalities. At Zoo Nuremberg, we planned the following days of intervention in advance: Days 2, 7, 15, and 30 postpartum, then once a month until 6 mo of age. Before intervention, the team meets and discusses all details. At the time of intervention, the calf should not have nursed for at least 20 min (to reduce risk of regurgitation). If the calf is too nervous, the intervention is stopped.

i. Details of the intervention:

(1) In shallow water at approximately 50 to 70 cm—If the animal is agitated, the water level is reduced.

(2) Position of the calf and mother (head to head) but separated by moveable walls (see Figure 3)—If the mother was pre-trained with frequent and positive human interaction, this physical separation might

Figure 3. Separation walls between mom and calf, if needed

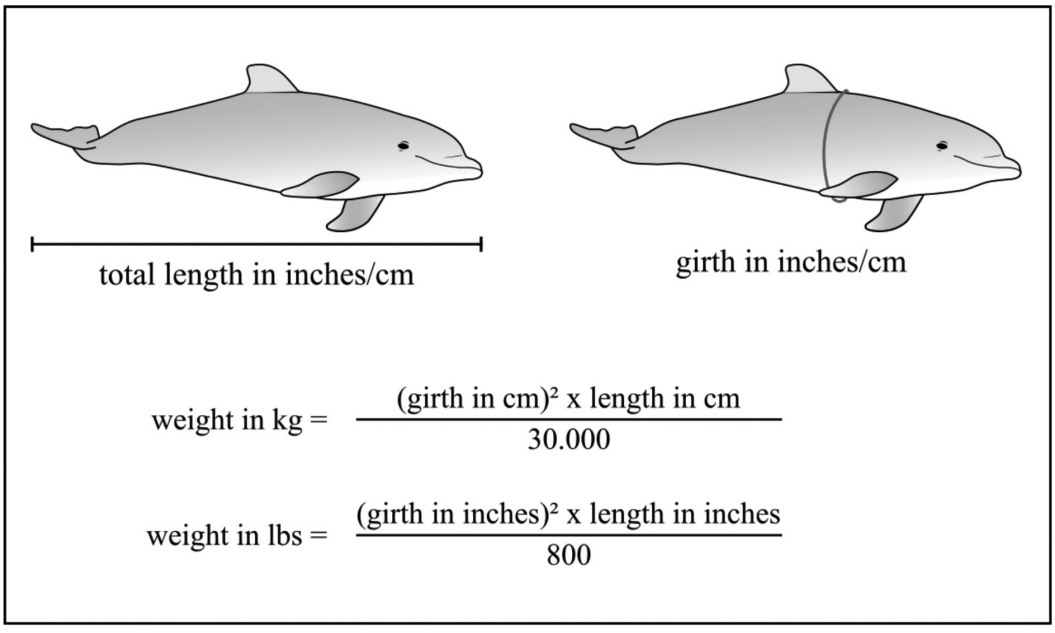

Figure 4. Weight formula measurement details

not be necessary or advisable in some cases.

(3) A calf-team is established—One person is responsible for measuring the heart rate (90 to 120 beats/min are ideal), one the breathing frequency (three to six breaths/min are ideal), and two others for holding the animal.

(4) All the data are registered on the "calf sheet."

(5) Weight is determined via morphometric formula (see Figure 4):

(girth in cm)² × length in cm/30.000 = weight in kg

(girth in inches)² × length in inches/800 = weight in pounds

(6) In the first 2 d, calves often lose some weight. From Day 3 on, they should gain weight again. The so-called "peanut head" is visible in the first days, but it should disappear between Days 7 and 10. The calf gains approximately 200 grams/d (Sweeney et al., 2010).

(7) Weighing on the scale—This is done only when the calf is used to being taken out of the water.

(8) Blood is taken and the glucose analyzed immediately on the "floating device" (see Figure 1).

(9) If the value is below 70 mg/dl, the calf is supplemented with formula, which is always prepared (milk team).

(10) Chlorhexidine can be used to disinfect the umbilicus.

(11) If constipation is suspected, fecal samples can be taken with a small tube, a syringe, and paraffin oil.

(12) Blowhole samples should be done routinely and analyzed bacteriologically as well as mycologically.

(13) Acoustic measurements are useful (Wells, 2009).

(14) Emergency drugs are always available:

- Atropine, 0.2 mg/kg BW
- Prednisolone, 1-10 mg/kg BW
- Adrenaline, 1:1,000, 1 ml mixed with 9 ml NaCl 0.9% ~ 0.1 ml /kg BW
- Dopram, 20 mg/ml, 0.5 to 1 mg/kg BW
- Bottle of oxygen

ii. At the end of the intervention, the moveable walls are moved away from the animals. The calf is always released first, with the mother released a few seconds later.

Comments: For a female calf at Zoo Nuremberg, we began intervention after 24 h and continued it on a daily basis due to small skin lesions. We always took blood and measured the calf using the formula for weight determination. The glucose level was 91 mg/dl on the first day and then between 118 and 181 mg/dl in the following blood samples (23 samples in 5 mo). The calf continuously gained weight. It weighed 10.7 kg on Day 1, 17.7 kg on Day 10, 27.15 kg at 1 mo, and 34.4 kg at 2 mo. The procedures took between 3 and 5 mo. This was possible because the various teams were well-trained.

c. Immunity—As the immunologic status of newborns is very low, and they depend entirely upon colostrum as their sole source of immunity (Sweeney et al., 2010), antibody use should be considered (if available). A dose of 1 mg/kg has given good results (Torsten Mölle, pers. comm.). Antibiotic treatment may be considered as well (e.g., Convenia, 6.7 mg/kg; continue for 10 d); and, in some cases, should be given high priority in treating neonates (Sweeney et al., 2010).

Comments: Due to signs of infection in the calf (blood values), antibiotics were injected daily.

d. Gating—This should be done as soon as possible, after 3 d and not later than 1 wk. Later on, calves are very reluctant to gate, even when the mother calls them.

e. Introduction to the other dolphins—A decision should be made on how and when to introduce mother and calf to the group. This depends on the group structure and the presence of experienced or young females (Mann et al., 1999).

Comments: At Zoo Nuremberg, the other members of the group were introduced gradually, starting with the oldest male. Introduction was conducted according to knowledge of the trainers. The "best friend" of the mother and the old male were the first to swim with the calf, followed by the other females, and finally the younger males.

Conclusions

Breeding bottlenose dolphins is a mandate of zoos, and reproduction has become very successful during the last two decades, creating a self-sustaining population and allowing animals under human care to experience natural behavior and live in social groups. Another aspect of reproduction is its important role in the well-being of the animals. Parturition and nursing are essential needs and important activities to keep our animals stimulated and active. This birth protocol is a result of years of experience in managing bottlenose dolphins, scientific research, and the advances in marine mammal medical knowledge. It includes all steps of parturition, birth, and the mother–calf relationship, and it is a very useful tool in the preparation of breeding dolphins. Various eventualities must be considered beforehand since the management of emergencies and unexpected situations require advance preparation. By pointing out the different phases of reproduction and addressing the practical applications, this protocol has proven to be highly effective in decision making and could help enhance dolphin reproduction in similar situations at other facilities.

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