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Control of fetal development in the female Black Sea bottlenose dolphin (Tursiops truncatus ponticus Barabash, 1940) using ultrasound and studying the dynamics of the linear dimensions of the chest of the fetus.

Semenov V.A.[1]* , Rodin I.A.[2], Vinokurova D.P.[2], Okolelova A.I.[2], Gavrilov B.V.[2]

[1]CJSC Gelendzhik Dolphinarium, 130 Lunacharsky St., Gelendzhik, Krasnodar Territory, 353460, Russia.
[2]Kuban State Agrarian University named after I.T. Trubilina, 13 Kalinina St., Krasnodar, Krasnodar Territory, 350044, Russia.

ABSTRACT

Pregnancy is an important period in the life of mammals, including cetaceans, held in captivity. This, of course, leaves its mark on the nutritional characteristics of animals, their mobility, the opportunity to participate in demonstrational events or scientific researches. Therefore, timely diagnosis of pregnancy, the establishment of a period and characteristics of the development of embryos in various periods of prenatal ontogenesis are extremely important. As a result of our studies, we were able to determine the gestational age in female bottlenose dolphins using their ultrasound examination in the embryonic and fetal periods, to reveal dynamic differences in the size of the fetal chest in the period from the third to twelfth months of pregnancy. However, these measurements had no significant differences between the group of females who gave birth safely on the one hand, and those who gave birth unfavorably on the other. At the same time, they revealed significant differences in hematological parameters. Trends in blood counts such as decreased hemoglobin, high alanine transaminase levels, and a large relative number of stab neutrophils can serve as markers of an unsuccessful pregnancy. Revealed a relatively young age (11,0 ±1,53 years) dysfunctional females, which turned out to be significantly ($P < 0,05$) below the age (15,8 ± 1,37years) had safe births.
Thus, the hematological parameters of pregnant females and their age can give an objective assessment of the probability of physiologically normal delivery.
Keywords: pregnancy, ultrasound, hematological parameters, bottlenose dolphin.

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Corresponding author

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INTRODUCTION

Pregnancy is an important period in the life of mammals, including cetaceans, held in captivity. This, of course, leaves its mark on the nutritional characteristics of animals, their mobility, the opportunity to participate in demonstrational events or scientific researches. Therefore, timely diagnosis of pregnancy, the establishment of a period and characteristics of the development of embryos in various periods of prenatal ontogenesis are extremely important (Shantiz A.Yu., 1999).

The gestation period for bottlenose dolphins lasts approximately 12 ± 1 months. For example, in the Indian bottlenose dolphin (T.t. Aduncus) with known dates of conception, a period of 370 ± 11 days is determined (Brook, 1997). The lactation period in bottlenose dolphins can last up to 2 years and even more in wild animals. (Robeck T.R. e. A., 2001). Most female bottlenose dolphins (cows) first produce calves between the ages of 7 and 10 years (Duffield et al., 2000). The full reproductive cycle, or the period of time between births, is 3-4 years. The duration of the estrous cycle in T. truncatus is 21 to 42 days (Sawyer-Steffan and Kirby, 1980; Kirby and Ridgway, 1984; Schroeder, 1990).

All cetaceans are single-birthed animals in which, cases of multiple-births are very rare and range from 0.002% in finwala (Balaenoptera physalus) to 0.5% in beluga whales (Delphinapterus leucas). A positive evolutionarily acquired trait, and rare cases of multiple pregnancy are classified as atavistic traits (Yablokov A.V. et al., 1972). According to available data (Ozharovskaya, 1997) about numerous cases of birth in bottlenose dolphins in captivity ended with the birth of one calf. Only two cases of double pregnancy are mentioned in the literature and none of them had a successful outcome. In one case, the twins were born dead (Ridgwey, Benirschke, 1977); in another, the cow had premature birth and four months after her death, at dissection, a full-term macerated fetus was found in her (Gray and Conclin, 1974).

A well-developed calf is born very large - from 1/4 to 1/2 the length of the mother's body. Rarely, several embryos are found in one female. Seven were once found in a blue whale, and six embryos in a finwal and a saival. This is evidence of the past multiple years of cetacean ancestry. Extra embryos usually dissolve, and twins can rarely be born (Tomilin A.G., 1957). The death of one of the embryos in humans can lead to serious health problems of the second or even its death. According to statistics, in the event of the death of one of the embryos in the first trimester of pregnancy, the probability of normal development and birth of the second reaches 90%. In this case, the embryo is completely reabsorbed or softened. If the death of one of the fetus occurred at a later date, then the second may develop severe damage to the central nervous system, internal organs or death (Tsivtsivadze EB, Novikova SV, 2014).

The reproductive function in dolphins, as in other mammals, is regulated by a number of neurological and hormonal mechanisms, involving the hypothalamus, pituitary, and sex glands. These three organs are commonly called the hypothalamic-pituitary-gonadal system (Aubin 2001).

The effect of various environmental factors on the reproductive cycle indicates that the reproduction process is under the control of nerve stimuli transmitted to the brain. The main part of the transmission and transformation of these stimuli appears to occur in the hypothalamus and its associated nuclei, the neurons of which secrete cryotropic hormones into the portal pituitary system (Robeck T.R. e. A., 2001). These hormones control the anterior pituitary, or adenohypophysis. Among such neurohormones is gonadotropin releasing hormone (GnRH), which is of great importance for the regulation of the endocrine aspects of mammalian reproduction. Activation of GnRH receptors in the anterior pituitary gland secretes luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. As the follicle grows and ripens, estrogen is produced due to the paracrine interaction between the cells of the inner membrane (theca interna) of the follicle and the granular layer (granulose) lining the follicle. Initially, an increase in estrogen synthesis inhibits both LH and FSH secretion in the pituitary gland. As the maturing follicle approaches the preovulatory stage, estrogen that has reached the maximum level of secretion begin to have a positive inverse effect on the frequency and amplitude of GnRH emissions, which in turn leads to preovulatory LH release. LH stimulates the formation of a low molecular weight glycoprotein, the so-called inhibin, in the follicle. Inhibin not only inhibits FSH secretion, but also increases the sensitivity of cells -theca interna- of the preovulatory follicle to LH (Baird and Smith, 1993). This combination of increasing the number of LH receptors and increasing sensitivity to LH provides an appropriate response to LH release and ovulation.

After ovulation, granular and parietal cells are converted into large progesterone-secreting and small luteal cells, respectively (Hendricks 1991). Progesterone, and to a lesser extent estrogen, produced by the corpus luteum, inhibits luteinizing and follicle-stimulating hormones, reduces the level of gonadotropin-releasing hormone released from the hypothalamus.

Therefore, until recently, the level of plasma progesterone was the most common criterion for pregnancy in the females of Black Sea bottlenose dolphins. Some authors consider this indicator a reliable diagnostic criterion if the level of progesterone in three consecutive samples with a 2-week break is higher than 3 ng / ml. (Schroeder 1990, Robeck et al. 2001). Unfortunately, in practice there are cases of pseudopregnancy, the causes of which are unknown to dolphins and can be numerous. In these cases, pregnancy cannot be confirmed without the use of ultrasound. The use of ultrasound to monitor pregnancy in captive cetaceans provides valuable data on the morphology, development, and well-being of the fetus. When examining the fetus, measurements of the chest and head are important, since they can play an important role in determining the gestational age of bottlenose dolphins (Williamson P., et al, 1990; Stone LR, EA, 1999; Semenov VA. Et al., 2016; Busharova E.V., 2011), however, as with other mammals, including humans (Medvedev M.V., 2005). Until 2008 (V. Semenov et al., 2008) in the territory of the Russian Federation, single attempts at ultrasound examination of Tursiops truncatus ponticus by practitioners on the Black Sea coast were not always successful, were not described in the literature and did not have a systemic character.

Assessment of the size of the chest and head are important parts of the examination of the fetus in dynamics to control its proper development. For example, due to the underdevelopment of the bone skeleton of the chest, lung hypoplasia can occur, which in turn can cause perinatal mortality in humans (Serov V. e.a., 1997; Saveliev, GM, 1998). At the same time, with reduced values of the diameter of the head, especially during full-term pregnancy, intrauterine growth retardation syndrome is often diagnosed, usually due to reduced utero-placental blood flow (Saveliev, GM E.A., 1991; Strizhakov AN, e.a. , 2004).

Female bottlenose dolphins kept in Atlantis Marine Park (Western Australia) used B-mode ultrasound to diagnose pregnancy and measure the growing fetus (Williamson et al. 1990). Examinations were performed at approximately a monthly interval using an AlokaSSD260 ultrasound machine (Aloka, Tokyo, Japan) with a linear sensor at a frequency of 33-5 MHz (AlokaUST-5021). The converter was enclosed in a thin plastic bag (to protect it against contact with salt water) and provided with a large amount of gel to ensure good contact with the object. Measurements were taken of the cross section of the chest at the level of the 2nd or 3rd intercostal space, the diameter of the skull, the width of the ribs and cervical vertebrae, as well as the length of the rostrum. Due to the short duration of the imaging process, proper images of the chest and the diameter of the skull (occipital-frontal axis) were obtained, images of the remaining structures of the fetus did not give reliable results. Sizing of the fetal structures was carried out by comparing them with a scale on the screen of the ultrasound apparatus. As a result of research, a linear dependence of an increase in the parameters of a developing fetus was revealed. The fetal heartbeat was regularly visualized and indicated fetal viability.

Of particular interest are studies of the growth dynamics of the head and chest of the fetus in cows with successful delivery on one hand, and unsuccessful delivery on the other hand.

Research objective

The purpose of these studies was to detect pregnancy in female bottlenose dolphins (Tursiops truncatus ponticus Barabash, 1940) using ultrasound and to study the dynamics of the linear dimensions of the chest of the fetus, as well as blood tests of pregnant cows , depending on its timing in cases of successful and unsuccessful delivery.

MATERIALS AND METHODS

In the period from 2007 to 2018, we detected 32 cases of pregnancy of the Black Sea bottlenose dolphins in 13 cows aged 6.5 to 23 years in various dolphinariums on the Black Sea coast and 149 studies were conducted. Due to the lack of experience in conducting an ultrasound examination of the Black Sea dolphins at the initial stages of studying this issue, not all studies turned out to be correct and reliable, therefore only 100 of them were included in this work and underwent biometric processing. The length of the animals varied from 240 to 275 cm, and the weight during the absence of pregnancy or in its initial stage ranged from 172 to

283 kg.

Pregnant animals require particularly gentle treatment, eliminating negative emotions that could harm the health of the mother and fetus. Therefore, the cow during the examination was not taken out of the water, but was examined at the side of the pool in a lateral lying position, which allowed her to raise her head above the water level at any time and to perform an unhindered act of exhalation-inhalation. In addition, dolphins hear each other's ultrasounds very well in water, as well as their reflections, which easily penetrate the body of these animals and reach the inner ear (Yablokov A.V., 1972). Therefore, it takes some time, perhaps several sessions with food reinforcement, so that the dolphin gets used to the ultrasound emitted by the sensor of the ultrasound apparatus, and at the same time does not show concern. These studies were focused on the detection of pregnancy using ultrasound examination of females and the study of the dynamics of the dorso-ventral (upper-lower) sizes of the fetal head and chest in the sagittal plane at different stages of pregnancy. The head was measured along the contours of the bones, and the chest, taking into account the soft tissues at the level of the heart. Pregnancy was established retrospectively from the date of birth.

All 32 cases of pregnancy were divided into two groups:

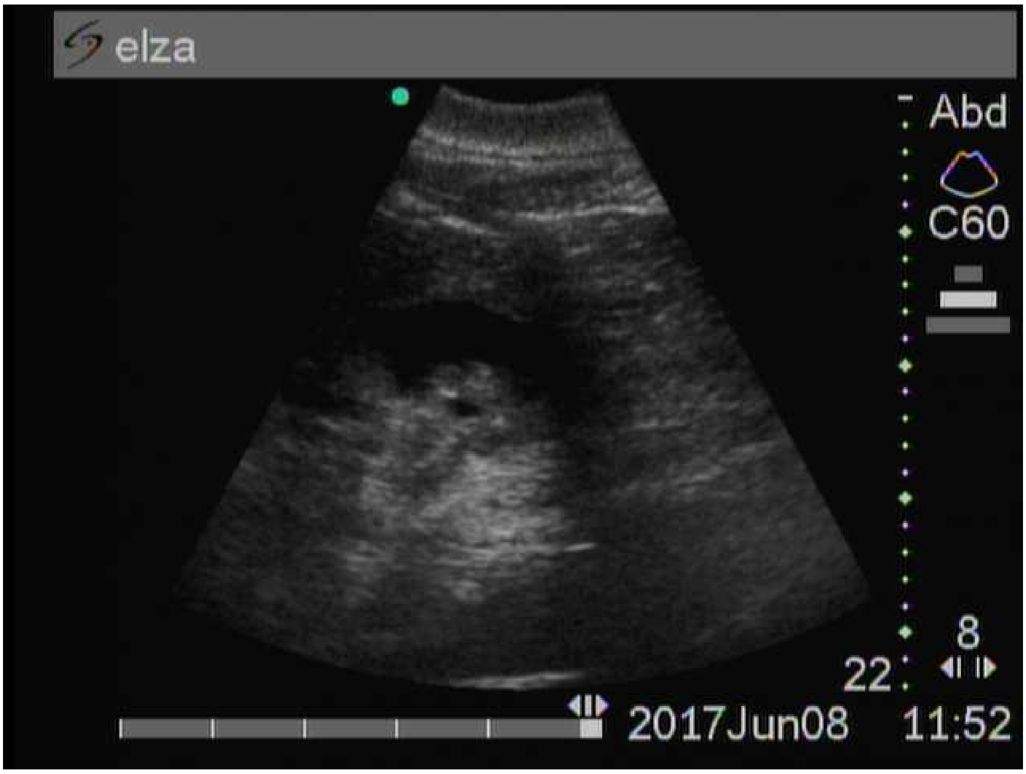
1) ended with a successful delivery;
2) ended in unsuccessful delivery, characterized by the birth of stillborn calves or those which died on the first day after birth.

Biometric processing of measurements of the fetus, as well as the results of blood tests of cows during pregnancy, was carried out both as a whole in all cases of research, and separately in each group. Ultrasound examination of pregnant cows was carried out using a SonoSite180 apparatus manufactured in the USA with a penetration depth of ultrasonic waves of up to 22 cm and a C60 / 5 - 2 MHz convex transducer. Blood for testing was taken from the tail veins Superficial fluke vv (Bossart GD, 2001). In this case, a clinical blood test was performed on a Cell-Dyn automated analyzer (Abbott USA) using Abbott USA test systems - a general blood test, biochemical studies were performed on an ARCHITECT C-8000 automatic analyzer (Abbott USA) using a test Abbott USA systems - biochemistry, the concentration of progesterone in the blood was determined using an Architect i2000 automated analyzer (Abbott USA) using Abbott USA test systems - hormones.

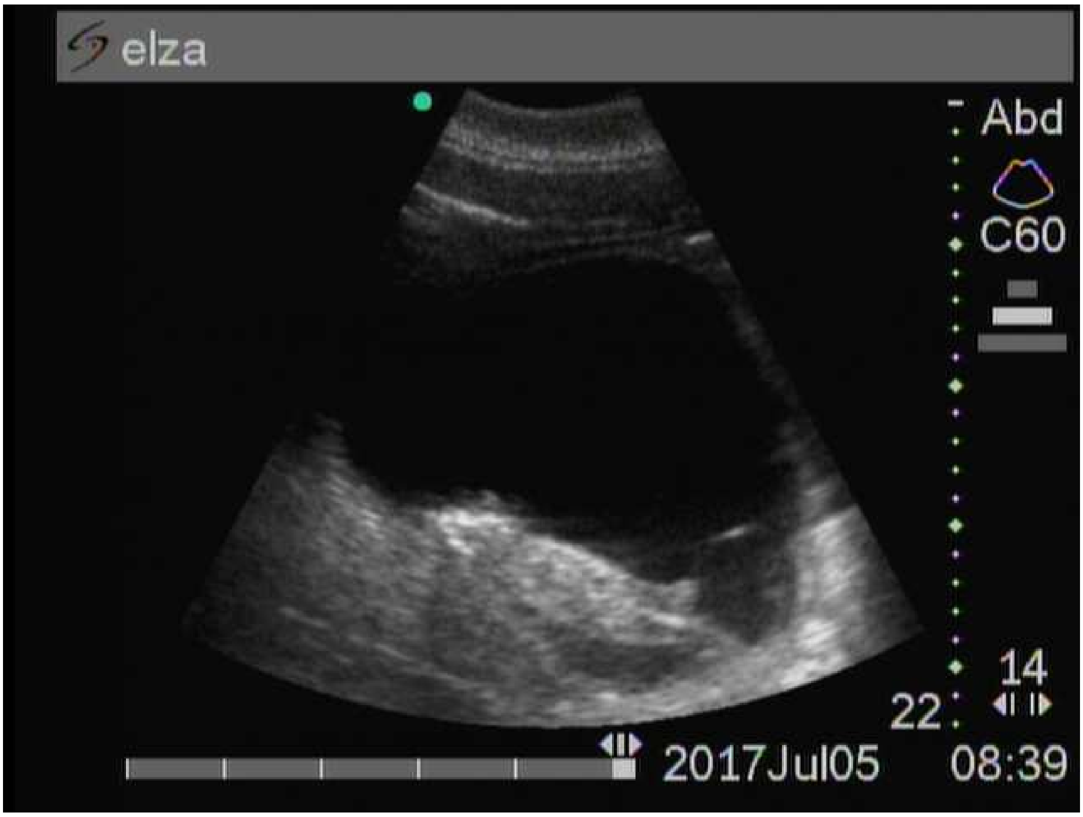
RESULTS AND DISCUSSIONS

In our studies, pregnancy in all cases ended with delivery through the natural birth canal. The level of plasma progesterone during pregnancy ranged from 1.70 to 120.0 ng / ml and was on average 24.0 ± 2.03 ng / ml (the number of studies was 82), while in the absence of pregnancy it was significantly lower ($P < 0.001$), varying from 0 to 17.1 ng / ml, and was on average 2.14 ± 0.392 ng / ml (the number of studies was 74). During the second half of pregnancy, fetal heart rate was recorded in all pregnant cows from 120 beats per minute at the 6th month and 117 beats per minute at the 7th month to 96 beats per minute at the beginning of the 12th month, and then up to 76 at the end 12th month 5 days before birth.

We managed to fix the earliest stage of pregnancy in a female bottlenose dolphin using ultrasound examination at the 2nd month of its existence (Figure 1). The level of progesterone in her blood at the time of the study was 7.58 ng / ml. A fetal egg was implanted in the right horn of the uterus. The figure shows that the hypoechoic uterine myometrium is lined with hyperechoic endometrium from the inside. The endometrium limits the anechogenic cavity of the chorion, in the ventral part of which the hyperechoic structure of the embryo is detected



At the 3rd month of pregnancy (Figure 2), the fetal egg has an upper-lower size of more than 10 cm. We see that the fetus is still small and is entirely placed in the scanning sector.



This figure shows the formation of the placenta, as well as the trunk, head, tail, hyperechoic spine. In this case, the fetal head was well identified as a separate anatomical formation, and the upper-lower size of the forming chest reached only 2.2 ± 0.31 cm (table 1).

Table 1. Dynamics of changes in the dorsal-ventral chest size (X ± m, cm) of the fetus during pregnancy in females of the Black Sea bottlenose dolphin.

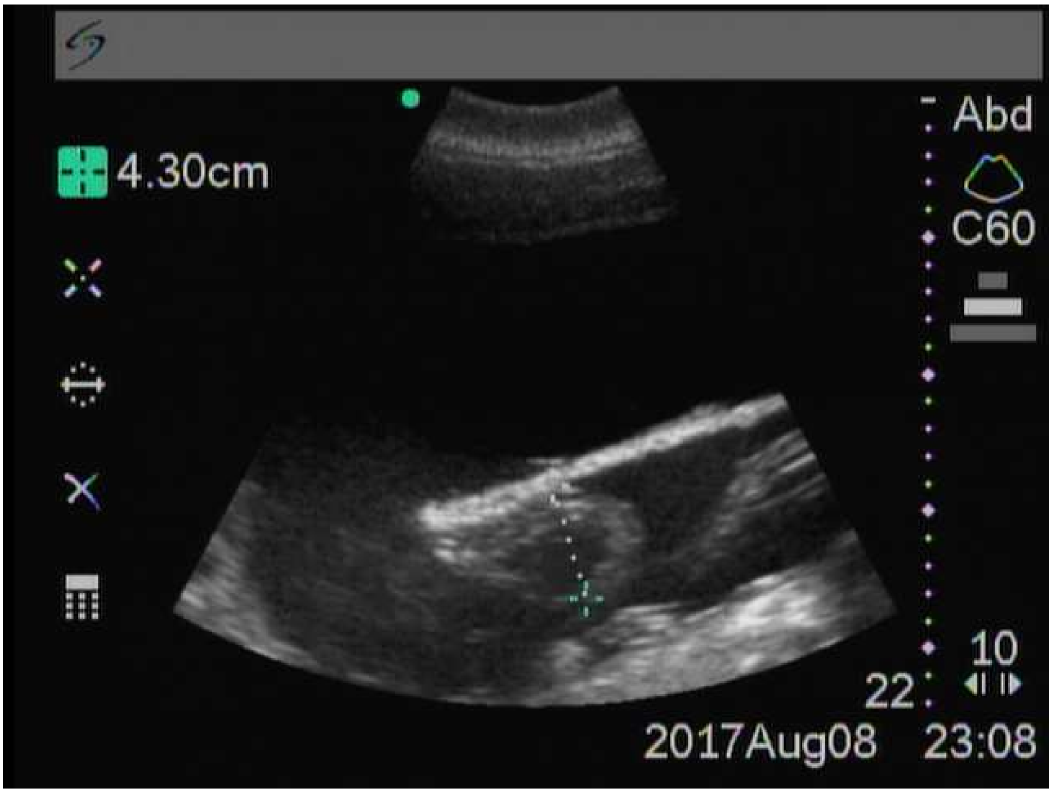
Pregnancy (months)	Normal delivery N=10, n=22		Pathological delivery N=7, n=10		All deliveries N=13, n=32	
	Number of studies	X ± m, см	Number of studies	X ± m, см	Number of studies	X ± m, см
3rd	4	2,2 ± 0,31	-	-	4	2,2 ± 0,31
4th	3	3,8 ± 0,13	1	3,85	4	3,8 ± 0,09**
5th	6	5,1 ± 0,12	1	5,29	7	5,1 ± 0,11***
6th	9	6,7 ± 0,22	2	7,0 ± 0,69	11	6,7 ± 0,21 ***
7th	11	8,0 ± 0,24	1	6,8	12	7,9 ± 0,24***
8th	11	9,7 ± 0,42	4	10,5 ± 0,75	15	9,9 ± 0,37 ***
9th	8	11,5 ± 0,33	1	11,3	9	11,5 ± 0,29 **
10th	11	13,0 ± 0,14	3	12,3 ± 0,29	14	12,8 ± 0,15 **
11th	10	14,4 ± 0,12	7	14,1 ± 0,29	17	14,3 ± 0,14 ***
12th	6	14,8 ± 0,22	1	15	7	14,9 ± 0,19*
Total	79		21		100	

Definition of parameters:

X - arithmetic mean; m -standard error for sample share;
N - the number of individuals examined; n –number of pregnancies studied
Significant differences between a given gestational age and a previous: *- P < 0,05; ** – P < 0,01; *** – P < 0,001

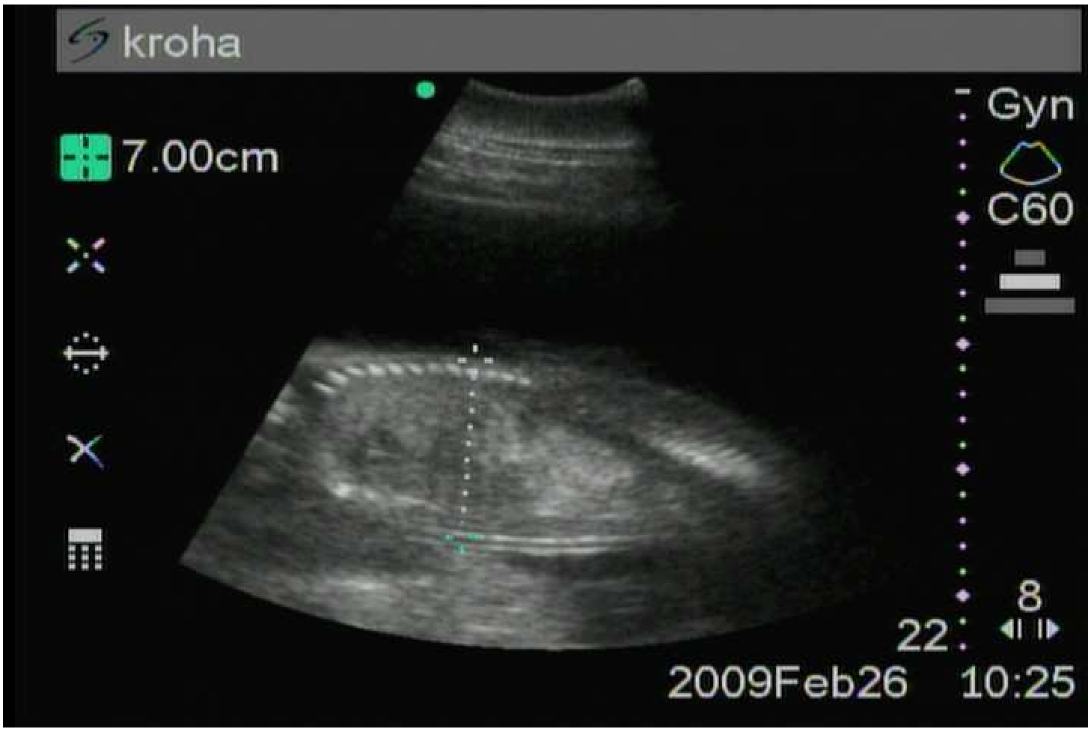
The same table shows that, starting from the fourth month of pregnancy and ending with the twelfth, with the increase in the size of the chest there is a high degree of reliability of the differences between this gestational age and the previous.

The largest relative increase in the chest of the fetus during the entire period of pregnancy took place in the fourth month, namely, by 72.7% (1.6 cm) compared with the previous and third, month. At this time, the ovum is significantly enlarged and extended, due to which we can observe a bend in the fallopian tube 1800 (Figure 3), in this case, the upper-lower size of the emerging chest is on average 3.8 ± 0.09 cm.



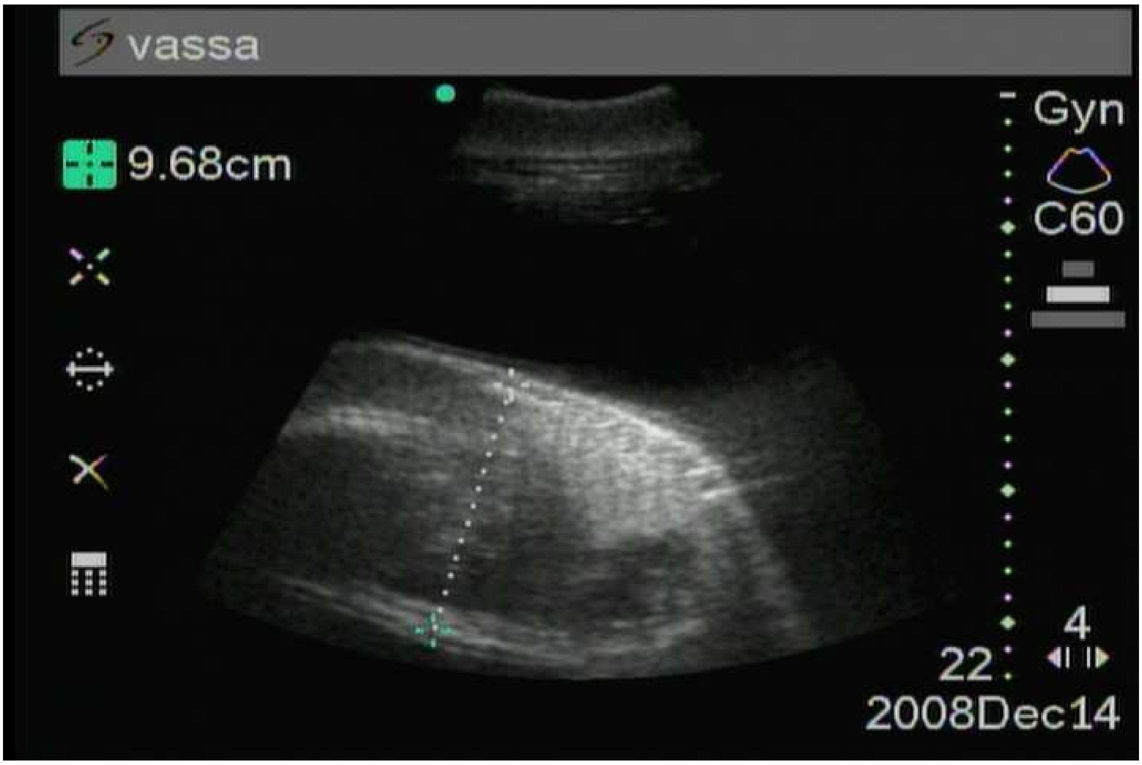
In the 5th month (figure 4), the fetus is actively moving in a large space of amniotic fluid. At this point there is an increase by 5,1 ± 0,11 cm. At this stage, it is possible to see the anechoic moving structure of the heart against the background of hypoechoic lungs, not filled with air, as well as the abdominal aorta and caudal vena cava in the form of anechogenic tubular structures

In the 6th month (figure 5), an increase in the dorso-ventral sizes of the chest appeared as in the 4th month, 1,6 cm (6,7 ± 0,21 cm) The heart is identified as an anechoic rounded motile structure. The heart rate reaches 117 beats per minute and it is constant in contrast to the physiological bradycardia of the newborn coming sometime after the first breath (Yablokov A.V., 1972) and taking place then throughout life. The fetal liver in the abdominal cavity is hypoechoic, due to which it is clearly visualized immediately after not straightened lungs in the chest cavity, which have higher echogenicity. The diaphragm between them can be located as a hyperechoic rim. Since amniotic fluid is present in the fetal stomach, it is defined as a rounded anechogenic structure adjacent to the liver. The spine is clearly visualized as a hyperechoic segmented structure that emits echo-acoustic shadows.



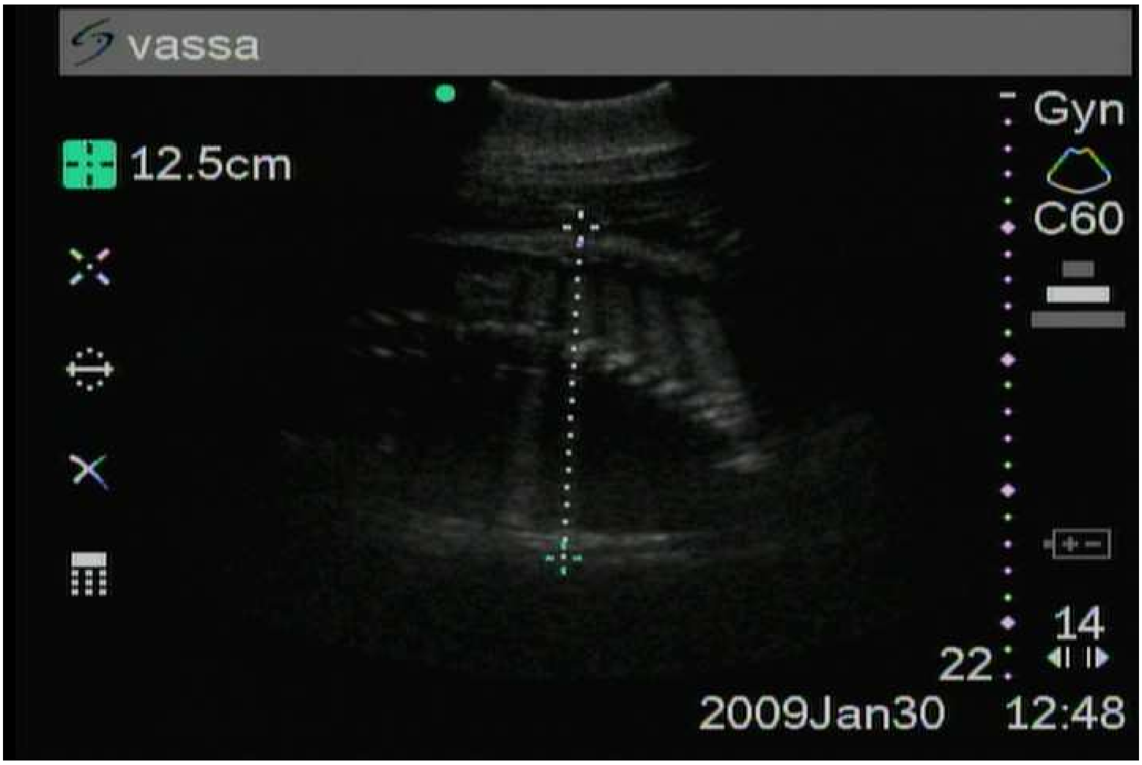
In the 7th month the size of the thoracic cavity of the fetus reached a size of 7,9 ± 0,24 cm (figure 6).

At the 8th month (figure 7), the diameter of the chest continues to increase significantly (P < 0.001) (by 2 cm) and reached a value of 9.9 ± 0.37 cm,

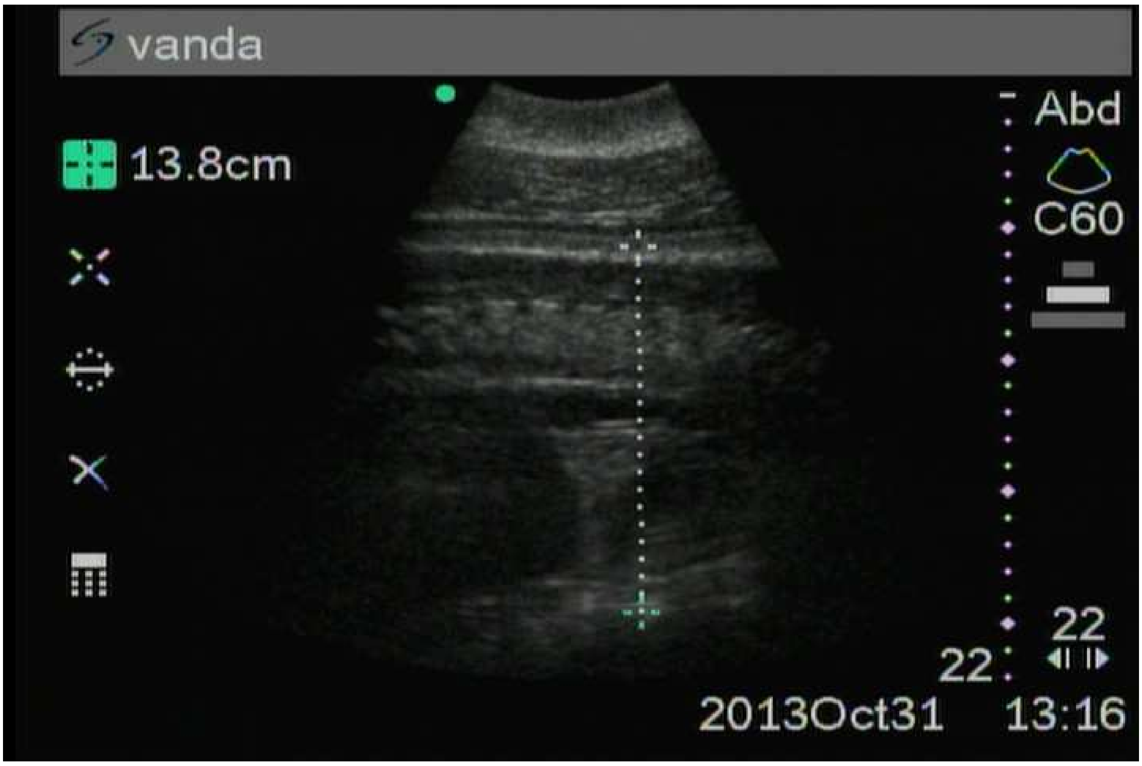


And in the 9th month (figure 8) the growth was 1.6 cm and averaged equal to 11,5 ± 0,29 cm.

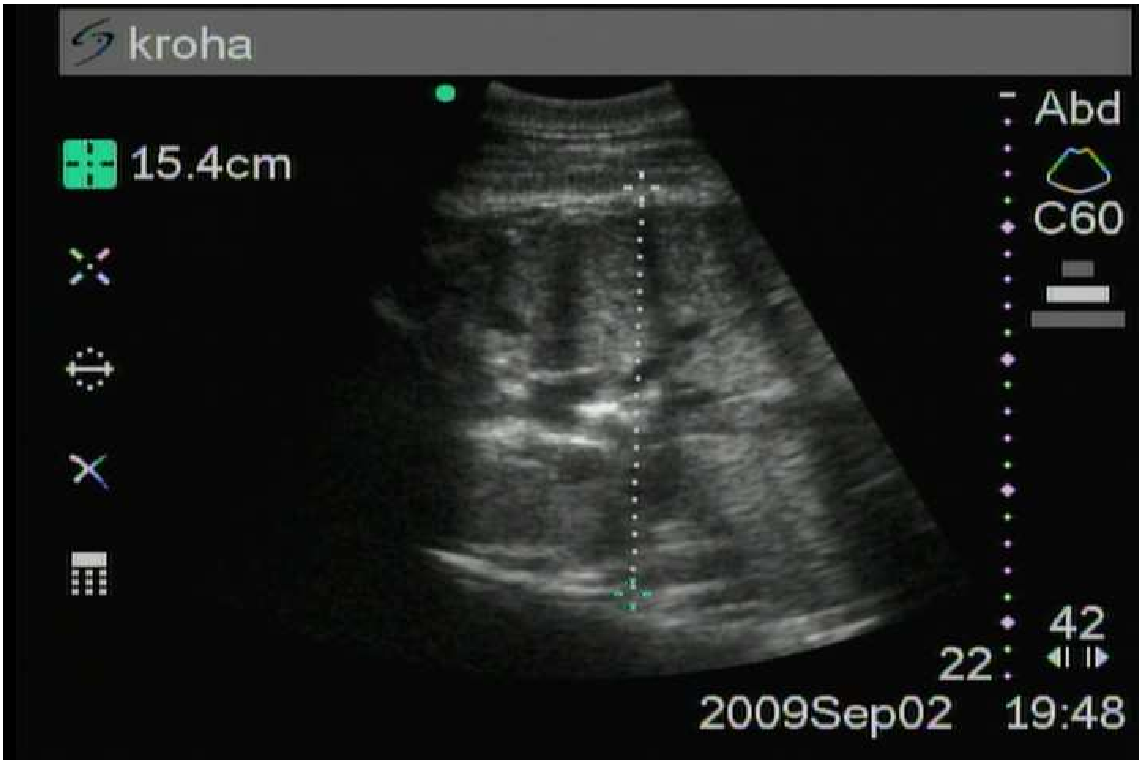
In the 10th month (figure 9) the thoracic cavity increased by1,3 cm and reached the diameter 12,8 ± 0,15 cm.



In the 11month (figure 10) the growth of the thoracic cavity was the same as in the 10th month (1,5 см) and the average was equal to 14,3 ± 0,14 см.



And finally, in the last month of pregnancy, the 12th month, chest size changed slightly by 0.6 cm, and its dorsal-ventral size averaged 14,9 ± 0,19 cm.



Thus, if at the 2nd month of pregnancy in the chorion cavity we noted hyperechoic structures of the embryo, then at the 3rd month we already find the fetus and placenta. The embryo becomes a baby with a cub-like configuration .The female is already undergoing a fetal period of pregnancy, which is characterized by rapid growth of the fetus, differentiation of tissues, the development of organs and systems from their primordia, the formation and development of new functional systems that ensure the life of the fetus in the womb and cub after birth.

Throughout the study period, there were cases of successful and unsuccessful delivery, which formed two groups of births. The individuals of the first group were safely born and lived for a long time. The second group included cases when the calves were born dead or died on the first day after birth. Of particular interest was not only a comparison of the chest measurements of the calves of the two groups during pregnancy, but also the blood components of their mothers at this point in life. From tables 2 and 3 it can be seen that no significant difference between the sizes of the chest at the same month of pregnancy were observed in these two groups of bottlenose dolphins.

Table 2. Hematological indicators (X ± m, см) during pregnancy in females of the Black Sea bottlenose dolphin

Indicators	Normal Delivery N=6, n=16		Pathological Delivery N=5, n=8		All Deliveries N=15, n=30	
	Q	X ± m, cm	q	X ±m, cm	q	X ±m, cm
Age(years)	16	15,8 ± 1,37	8	11,0 ±1,53*	24	14,2 ±1,13
Haemoglobin, g/l	85	172,8±1,15	42	164,5 ±1,70 ***	127	170,1 ±1,01
Erythrocytes, x 10^{12}/l	99	3,8±0,05	54	3,8±0,05	153	3,8±0,03
Leukocytes, x 10^{9}/l	99	7,7±0,18	54	7,3±0,30	153	7,6±0,16
Stab neutrophils, %	99	1,4±0,12	54	2,2 ±0,26 *	153	1,7±0,12
Segmented neutrophils,%	99	55,0±1,17	54	56,0 ±1,22	153	55,3 ±0.87
Eosinophils , %	99	19,0±0,74	54	17,7 ±1,10	153	18,5±0,62
Lymphocytes, %	99	20,9±0,92	54	21,3 ±1,23	153	21,1 ±0,74
Monocytes, %	99	3,4±0,19	54	3,0±0,29	153	3,2±0,16

ESR, mm/hr	90	3,0±0,28	52	3,1±0,47	142	3,0±0,25
Progesterone , ng/ml	49	24,4±2,98	33	23,4±2,47	82	24,0±2,03
Protein, g/l	72	69,7±0,74	38	70,2 ±1,12	110	69,9±0,62
Urea, mol/L	62	16,5±0,59	34	16,2 ±0,67	96	16,4±0,45
Creatinine, µmol/L	68	128,1±3,29	33	123,9 ±4,87	101	126,7±2,72
Glucose, mol/L	72	5,1±0,14	35	4,8±0,22	107	5,0±0,12
ALAT, U/L	77	55,8±5,06	40	88,5 ±19,77**	117	67,0±7,62
AST,U/L	78	291,0±20,90	40	373,3 ±70,67	118	318,9 ±27,70
GGT, U/L	74	35,0±1,88	37	37,0 ±2,90	111	37,7 ±1,58
ALP, U/L	67	508,5±46,41	34	673,8 ±70,71	101	564,2 ±39,49
Iron, µmol/L	69	28,2±1,15	41	26,4 ±1,28	110	27,5 ±0,87

Convention;

X – arithmetic average; m - the standard error for a sample proportion;
N –number of examined individuals ; n –number of pregnancy cases studied; *q –number of studies*
Significance of differences between a given gestational age and a previous: * – P < 0,05; ** – P < 0,01; *** – P < 0,001

From table 2 it can be seen that significant differences in hematological parameters between the group of safely giving birth to females and dysfunctional giving birth are noted primarily in the concentration of hemoglobin. In safe births, the amount of haemoglobin (P < 0,001) was significantly higher (172,8±1,15 g/L), than other cows with complicated births(164,5±1,70 g/L). At this given period, individuals of the first group had significantly (P < 0,01) lower levels of (55,8±5,06 units/L) alanine transaminase, than the animals in the second group(88,5 ±19,77 units/L) indicating a lack of toxicosis, hepatocyte integrity, and normal liver function. The relative contents of "stab" neutrophils (1,4±0,12 %) also turned out to be accurate (P < 0,05) if not a little below their counterparts with birth complications (2,2±0,26 %) in which a more pronounced "left shift" of the leukoformula was noted. This fact indicates a greater demand in the second group of animals for phagocytosis by neutrophils of tissue fragments and the destruction of opsonized microorganisms.

For other blood parameters, the difference was not significant. It can be noted that the group of normally giving bottlenose dolphins showed a lower level (508,5±46,41 U) Alkaline phosphate (ALP), than the other cows with birth complications (673,8 ±70,71 units/L). However, the difference in concentration of ALP, the level of which with age, bottlenose dolphins decrease from approximately 1300 to 200 units/Litre.rather indicates a relatively young age of dysfunctional females (9,8 ± 0,87 years), which turned out to be reliable(P < 0,05) below the age of normal birth (16.6 ± 1.20 years).

CONCLUSION

As a result of our studies, we were able to determine the gestational age in female bottlenose dolphins using their ultrasound examination in the embryonic and fetal periods, to reveal dynamic differences in the size of the fetal chest in the period from the third to twelfth months of pregnancy. However, these measurements had no significant differences between the group of females who gave birth safely on the one hand, and those who gave birth unfavorably on the other. At the same time, they revealed significant differences in hematological parameters. Trends in blood counts such as decreased hemoglobin, high alanine transaminase levels, and a large relative number of stab neutrophils can serve as markers of an unsuccessful pregnancy. Revealed a relatively young age (11,0 ±1,53 years) dysfunctional females, which turned out to be significantly (P < 0,05) below the age (15,8 ± 1,37years) had safe births.

Thus, the hematological parameters of pregnant females and their age can give an objective assessment of the probability of physiologically normal delivery.

REFERENCES

[1] Ozharovskaya L. V Morphology of the reproductive system of the black sea bottlenose Dolphin. / Black sea bottlenose Dolphin Tursiops truncatus ponticus: Morphology, physiology, acoustics, hydrodynamics / ed. - M., Science, 1997. P. 102 – 113.

[2] Ozharovskaya L. V. Reproduction of the black sea bottlenose Dolphin. / Black sea bottlenose Dolphin Tursiops truncatus ponticus: Morphology, physiology, acoustics, hydrodynamics / ed. - M., Science, 1997. Pp. 114-145.

[3] Semenov V. A., Danilova M. N., Smyshnov A.V. Ultrasound examination of female black sea bottlenose dolphins (Tursiops truncatus ponticus Barabash, 1940) during pregnancy. Proceedings of the sixth international conference "Marine mammals of the Holarctic". - Kaliningrad, 2008.

[4] Semenov V. A., Danilova M. N., Smyshnov A.V., Kozlov Yu. V., Osipova I. V., Vasiliev A. A., Shantyz A. Yu., Rodin I. A. Ultrasonic method for determining the presence and term of pregnancy in females of the black sea bottlenose Dolphin. / Patent for invention No. 2591730 / / Registered in the State register of inventions of the Russian Federation. - M., 2016.

[5] Tomilin A. G. Cetaceans / / Animals of the USSR and adjacent countries.- Publishing house of the USSR.- 1957, T 9. - 756c.

[6] Tsivtsivadze E. B. Novikova S. V. Multiple pregnancy: a modern view on the problem of pregnancy and childbirth (literature review) / Regular issues of "rmzh", No. 1, 2014, P. 16.

[7] Santis, A. Yu., Regularities of morphogenesis of the reproductive organs of male pigs in the prenatal and early postnatal ontogenesis / A. J. Santis. - Autoref. dis...d-RA Biol. sciences'. - Krasnodar, 1999. - 367 p.

[8] Yablokov A.V. Whales and dolphins / A.V. Yablokov, V. M. belkovich, V. I. Borisov. - M., Science,1972. - 472c.

[9] Aubin DJSt. 2001. Endocrinology. In: Dierauf LA, Gulland FMD, editors. CRC Handbook of Marine Mammal Medicine. Second Edition. Boca Raton. p 165-193 .

[10] Baird, D.T., and Smith, K.B., 1993, Inhibin and related peptides in the regulation of reproduction, in Oxford Review of Reproductive Biology, Milligan, S.R. (Ed.), Oxford University Press, Oxford, U.K., 191–232.

[11] Bossart G D., Reidarson T H., Dierauf LA and Duffield DA.2001.. Clinical Pathology. In: CRC Handbook of Marine Mammal Medicine / Dierauf L.A. and Gulland F.M.D., eds. Second Edition. Boca Raton: 593-620

[12] Brook, F., 1997, The Use of Diagnostic Ultrasound in Assessment of the Reproductive Status of the Bottlenose Dolphin, Tursiops aduncas, in Captivity and Applications in Management of a Controlled Breeding Programme, Ph.D. thesis, Hong Kong Polytechnic University, Kowloon, Hong Kong, 339 pp.

[13] Brook F, Bonn WV, and Jensen ED. 2001. Ultrasonography. In: Dierauf LA, Gulland FMD, editors. CRC Handbook of Marine Mammal Medicine. Second Edition. Boca Raton. p 593- 620.

[14] Duffield, D.A., Shell, D., and Dudley, M., 2000, Demographic analysis of breeding bottlenose dolphins in North American zoological facilities: 1976–1998, in Report from the Bottlenose Dolphin Breeding Workshop, Duffield, D.A., and Robeck, T.R. (Eds.), American Zoological Association Marine Mammal Taxon Advisory Group, Silver Spring, MD, 139–156.

[15] Gray K.N., Conclin R.H. Multiple births and cardiac anomalies in the bottlenosed dolphin // J. Wildlife Diseases. 1974. Vol. 10.P. 155 – 157.

[16] Hendricks D.M., 1991 , Biochemistry and physiology of the gonadal hormones, in Reproduction in Domestic Animals, Cupps, P.T. (Ed.), Academic Press, San Diego, CA: 81-118.

[17] Kirby, V.L., and Ridgway, S.H., 1984, Hormonal evidence of spontaneous ovulation in captive dolphins (Tursiops truncatus and Delphinus delphis), Rep. Int. Whaling Comm., Spec. Issue 6: 459–464.

[18] Lacave, G., M. Eggermont, T. Verslycke, F. Brook, A. Salbany, L. Roque and R. Kinoshita. 2004. Prediction from ultrasonographic measurements of the expected delivery date in two

species of bottlenose dolphin (Tursiops truncatus and Tursiops aduncus).Veterinary Record 154:228–233

[19] Ridgwey S.H., Benirschke K. Breeding dolphins: Present status, suggestions for the future: Final rep. to US Marine Mammals Comm. Wash. (D.C.), 1977. 308 p.

[20] Robeck TR, Atkinson SKC, Brook F. 2001. Reproduction. In: Dierauf LA, Gulland FMD, editors. CRC Handbook of Marine Mammal Medicine. Second Edition. Boca Raton. p 193-226 .

[21] Robeck TR, Schmitt TL and Osborn S. 2015. Development of predictive models for determining fetal age-at-length in belugas (*Delphinapterus leucas*) and their application toward *in situ* and *ex situ* population management. Marine Mammal Science. *Volume 31, Issue 2,* p. 591–611.

[22] Sawyer-Steffan, J.E., and Kirby, V.L., 1980, A study of serum steroid hormone levels in captive female bottlenose dolphins, their correlation with reproductive status, and their application to ovulation induction in captivity, National Technical Information Service, PB80-177199, Springfield, VA.

[23] Schroeder, J.P., 1990, Reproductive aspects of marine mammal., In CRC Handbook of Marine Mammal Medicine, Dierauf, L.A. (Ed.), CRC Press, Boca Raton, FL, 353–369.

[24] Savelyev G.M ., Fedorov M.V., Klimenko P.A., 1991, LG Sichinava Placental insufficiency . Moscow, Medicine , 400 .

[25] Saveliev G.M. , 1998, Ways to reduce perinatal morbidity and mortality. Bulletin of the Russian Association of Obstetricians and Gynecologists, 2 : 29-31 .

[26] Semenov V.A., Danilova M.N., Shantyz A.Y., Udovichenko L.D., Smyshnov A.V. and Osipova I.V. 2016. Prenatal Ultrasound Dating in Black Sea Bottlenose Dolphins. The Aquatic Veterinarian, 10(1): 20-23.

[27] Serov V., Strizhakov A.N., Markin S.A., 1997, Guidance on the Practical Obstetrics . Moscow: Medical Information Agency Ltd, 424 .

[28] Strizhakov A.N., Davydov A., Belotserkovtseva L.D., Ignatko I.V., 2004, Physiology and pathology of the fetus - Moscow, Medicine , 356 .

[29] Williamson P., Gales N.J., and Lister S., 1990, Use of real-time B-mode ultrasound for pregnancy diagnosis and measurement of fetal growth rate in captive bottlenose dolphins (Tursiops truncatus), J. Reprod. Fertil., 88: 543–548.