

Evaluating the Efficacy of Ultrasonographic and Macroscopic Approaches for Pregnancy Diagnosis in Golden Hamsters

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Abstract

The hamster is a popular experimental model for studying the reproductive system. However, it has always been challenging to diagnose Pregnancy and aging. In certain tiny laboratory animals, such as rabbits, rats, along with mice, ultrasonography has been used to diagnose pregnancy. The trans-abdominal ultrasound used to diagnose pregnancies and determine fetal ages in golden hamsters. Ten mature female golden hamsters were chosen and housed in a 14-hour light/10-hour dark cycle. Electrocardiogram (ECG) and Human chorionic gonadotropin (HCG) were used to synchronize the estrous cycle. After seven days after mating, a medical ultrasound machine equipped to use a linear 8.5 MHZ probe was used to check the hamsters every day for signs of age and pregnancy. The pregnant hamsters was put to death each day of the experiment and then dissected to obtain macroscopic fetal measures with a digital caliper. The Objective of the study was to evaluating the efficacy of ultrasonographic and macroscopic approaches for pregnancy diagnosis in golden hamsters.

Keywords: Hamster, pregnancy, Human chorionic gonadotropin, Electrocardiogram Macroscopic.

Introduction

Cesarean scar defects (CSD) can range from a little weakness of the surface myometrium to a serious defect or niche⁴ due to the remodeling of the lower uterine region following cesarean delivery (CD). The procedures employed for hysterotomy closure and scar placement both have an influence on the incidence of CSD, which increases with the number of previous CDs (1). Women over 35 who get pregnant are referred to as having an "Advanced Maternal Age (AMA)." Around the world, more women are developing AMA every year. The number of expecting mothers with AMA who was prepared to undergo Non-invasive Prenatal Testing (NIPT), which may determine the most common fetal aneuploidies (trisomy 21, trisomy 18, together with trisomy 13), while sex chromosomal aneuploidies (SCAs) has increased in recent years. However, many of these women still choose invasive testing (2). Although ultrasound has been extensively used to evaluate human pregnancies, studies of animal pregnancy have not utilized ultrasound as frequently. Ultrasound has only lately been used on non-rodent animals. In animal studies, abdominal palpation is a pregnancy diagnostic method that should only be applied in mid-to-late pregnancy. The human reproductive system usually uses the polytocous hamster as an experimental model because of its short gestation time (3). Unfavorable pregnancy results from the potential of specific inflammatory cytokines to cause excessive inflammation via related receptors, despite the fact that a controllable inflammatory response is essential in the pregnancy process. Over the uterine-placental interface, immune

cells have a profound impact on pregnancy. Local immune cells prevent immunological tolerance to fetal antigen from developing, non-infectious inflammatory response, and preserve pregnancy (4). Due to the special biochemical, physiological and immunological changes women must endure to accept the fetus, and pregnancy increases the risk of a variety of viral infections and the severity of linked disorders. Pregnant women contracting viruses frequently experience serious or deadly consequences such as intrauterine growth restriction, premature delivery, stillbirth, or miscarriage (5). High-frequency sound waves are used in ultrasonography to the inside of organs. It is a safe and effective way to diagnose pregnancy in various animal species, including rats (6). The macroscopic approach, also known as abdominal palpation, is used to physically inspect the golden hamster's belly for pregnancy-related changes. Compared to ultrasonography, the visible process needs little equipment and is reasonably priced (7). To identify changes suggestive of pregnancy, abdominal palpation includes gently rubbing the hamster's belly with your fingertips. Usually, 10 to 14 days after mating, the growing embryos and the larger uterus can be felt as little bumps or a hard mass in the belly during the latter stages of pregnancy (8). To improve precision and dependability, scientists and veterinarians that combine with golden hamsters frequently combine these techniques. These techniques can be used with hormonal analyses, such as testing progesterone levels, to confirm pregnancy (9). A skilled examination can detect a variety of pregnancy symptoms, such as changes in the size, shape, and consistency of the reproductive organs. Additionally, it could spot alterations in the mammary glands and fetal secretions in the vagina (10).

The period between copulation and parturition in animals that are not prone to a delay in either fertilization or implantation indicates how long the gestation lasted since the timing of copulation and ovulation is often and continuously correlated (11). Study (12) provides the trans-abdominal ultrasound used to diagnose pregnancies and determine fetal ages in golden hamsters. The cross-sectional observational study examined the connection between pre-pregnancy obesity and overweight, periodontitis during the third trimester of pregnancy, and the infants' birth weights. To establish whether the right and left sides of a tubal pregnancy (TP) have different clinical features and whether there were any left-right asymmetries or variables that impact lateral dominance (13). The study (14) evaluate the sensitivity of ultrasound imaging in an outpatient community setting in detecting asymptomatic microscopic hematuria (AMH) among patients with upper urinary tract cancer. Study (15) have the effect of uterine pre-sensitization with seminal antigens during breeding on the success of cow pregnancies was investigated. Cows who were lactating were given 0.5 mL seminal plasma (SP), 40 ng recombinant human transforming growth factor-1 (rhTGF-1), or 0.7 mL bovine serum albumin (BSA) before or at insemination, or they were left untreated (n = 1092 for beef cows and 800 for dairy cows). The micronucleus (MN) test is being used in the study to determine whether DNA damage and issues during pregnancy or infertility in persons are linked. It provides a quick synopsis of the research that has been published to date(16). The goal (17) was to learn more about Total cholesterol (TC) and triglycerides (TG) plasma concentrations increase physiologically during pregnancy as a result of increased insulin resistance, estrogens, progesterone, and placental lactogen. Although their reference

values were not precisely known, TG levels could rise to 300 mg/dL, and TC levels can reach 350 mg/dL. They aimed to evaluate the efficacy and safety for non-invasive thrombotripsy using a robotic-assisted along with high-frequency ultrasound approach to recanalize proximal deep venous thrombosis (DVT) in a pig model (18).

Materials and Methods

The trials were overseen by the Prevention of Cruelty to Animals, and carried out in conformity with Iranian animal ethical guidelines (19).

Wildlife

A total of 10 adult hamsters were chosen, and they were housed in separately ventilated cages between 22 and 23 °C, with 15 hours of light along with 11 hours of darkness (20). They were fed typical laboratory unrestricted water availability and a mouse diet. Visual observation is one of the most frequently used to identify pregnancy in golden hamsters. The female hamster will likely put on weight as the pregnancy goes on and have a rounder belly. To determine whether the hamster is pregnant, closely examine the physical changes to their body and contrast them with how she usually appears. This approach, meanwhile, isn't always accurate, particularly in the first trimester. It's crucial to remember that while these symptoms may suggest pregnancy, they are not always present and might differ between different hamsters. Specialized methods like ultrasound imaging can be employed for a more precise diagnosis, particularly in research or breeding situations. Speaking with a vet or an experienced hamster breeder is advised for an accurate pregnancy diagnosis in golden hamsters.

Synchronizing the ovaries, doing an ultrasound, and performing a macroscopic assessment

Female hamsters go via the estrous cycle four times every week. Superovulation was induced with five units of intraperitoneal ECG. After 54 hours, HCG (5 units) was injected to promote ovulation. Several hours following the HCG injection, vaginal discharge—which indicates estrus—occurs (21). Hamsters produce a creamy, sticky release in the morning following ovulation that has a distinct odor. The vaginal smear contains a large number of cornified epithelial cells. The female hamsters' vaginal releases were checked to make sure they had a regular estrous cycle prior to mating. Injecting 0.3 cc of normal saline into vagina and also collecting it using pipette, vaginal lavage was carried out to ascertain the timing of estrus (22). Care was given to prevent cervical stimulation when inserting the pipette along with flushing the vagina to avoid doing so too deeply. Vaginal smears were examined using light microscope to detect cells at a magnification and to quantify the fraction of cells. After the HCG injection during the estrus phase, men and women were combined for 18 hours in a 1:1 mating ratio (23). The first day for pregnancy was regarded as the day sperm were discovered in vaginal smears. The embryonic vesicle's size, echogenicity, and head diameter, with length from the crown to the rump, were used to calculate the results of the transabdominal ultrasound test from the time of mating until day 17 of pregnancy. The hamsters were

manually held at the dorsal recumbent position. To avoid endangering the unborn child, anesthesia was not utilized during the ultrasound test. The first hamsters received Ketamine-Xylazine to render them unconscious during the ultrasound examination for each day of pregnancy detection. Ten cervical dislocation surgeries were done under heavy anesthesia. To reach the embryos, the uterus had to be removed. A digital caliper was used to measure the diameter of the embryonic vesicle, the head, and the length of the crown-rump. The practice of stimulating the production of numerous follicles in the ovaries to increase the likelihood of successful fertilization and pregnancy is known as synchronizing the ovaries. This procedure is commonly combined with assisted reproductive technologies such as intrauterine insemination (IUI) or in vitro fertilization (IVF). The number of viable eggs available for fertilization can be enhanced by synchronizing the ovaries so that many follicles mature at the same time.

Statistic evaluation

The data were statistically analyzed with Statistical Package for the Social Sciences (SPSS) software together with the Pearson correlation test. The information was displayed as mean. P-values under 0.06 were regarded as significant. The sensitivity represents the proportion of pregnant hamsters that are correctly diagnosed. It is calculated by dividing the total number of true positives and false negatives by the number of true positives. A high sensitivity shows that the diagnostic approach is successful in detecting pregnant hamsters and predicts a low number of false negatives.

Results

The earliest diagnostic indicator of pregnancy during an ultrasound scan was the gestational sac diameter which could only be accurately assessed on day 8 of pregnancy. The fetal vesicle's average size varied from 11.7 mm on day 9 to 28.6 mm on day 19. From day 12 of pregnancy, one may measure the head's circumference. On day 7 during pregnancy, the average head diameter had been 5.152.48 mm, and on day 17 of pregnancy, it was 10.211.58 mm. The length from the crown to the rump was 7 mm on day 8, with 24.56 mm at day 18 during pregnancy.

Table (1): Ultrasonographic (US) and macroscopic (Mac) evaluation of golden hamster fetuses and gestational sacs on days 7-17 of pregnancy

Day	Number of fetus (Mac)	CRL (mm;Mac)	HD (mm;Mac)	Number of fetus (US)	GSD (mm;US)	GRL (mm;US)	HD (mm;US)
8	5	7.18 ± 0.68	4.1±1.41	4	11.2±0.0	9±1.1	
9	6	8.03±0.66	5.3±1.15	3	13.5±0.66	8.97±0.41	
10	6	9.67±0.72	6.2±0.36	4	12.4±1.01	9.55±0.66	

11	4	9.5±1.27	5.7±0.7	3	13.67±0.17	10.31±1.19	6.27±0.58
12	5	21.85±1.09	6.21±0.92	4	16.94±0.24	21.4±1.53	5.93±0.6
13	5	12.51±1.97	5.69±0.81	2	15.76±0.68	41.8±3.9	7.2±0.7
14	4	14.52±1.72	7.01±0.7	2	19.2±0.0	16.1±0.0	8±0.0
15	3	17.97±0.33	8±0.55	3	17.89±0.39	17.6±1.72	8.99±1.03
16	5	28.55±0.71	9.04±1.7	3	22.1±0.0	19.99±0.0	8±0.0
17	7	30.18±0.74	8.4±0.57	2	23.44±0.62	21.9±0.54	14.6±0.38
18	4	24.95±0.81	9.3±1.87	3	26.2±2.7	26.65±1.02	20.61±1.89

The macroscopic examination for the fetus in golden hamsters from day 8 to day 18 of pregnancy is represents in (Figure 1). Crown-rump length (CRL) along with head diameter (HD) was among the indicators employed. Beginning on day 7 of pregnancy, it was possible to measure the fetus's head diameter.

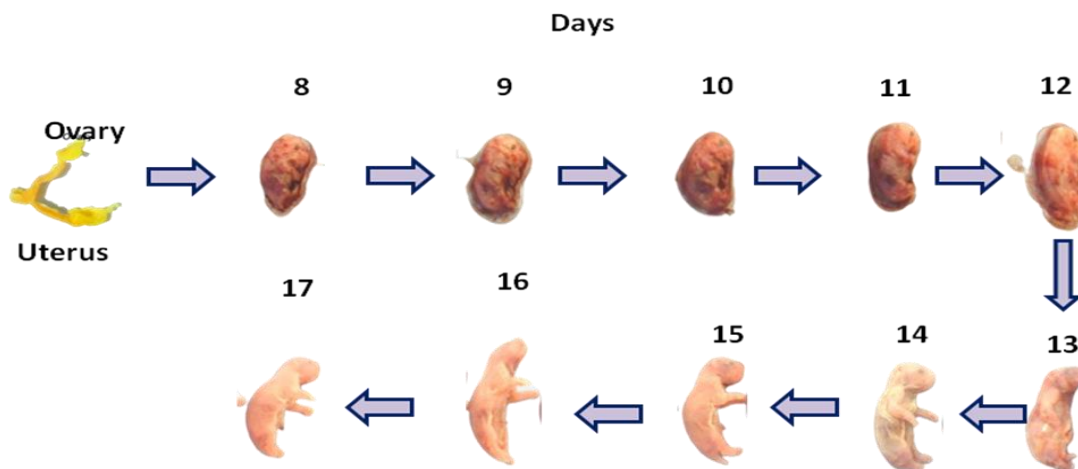


Figure (1): Examination of the fetus in golden hamsters from day 8 to day 18 of pregnancy

During pregnancy, an average head diameter was 3.10.32 mm and 9.31.78 mm on days 7 to 17, respectively. The length from the crown to the rump measured 6.190.48 mm with 24.950.91 mm on days 8 and 17, respectively, of pregnancy. There are 28 of 58 fetuses found during the ultrasound scan were also removed from the uteri. The association among days of pregnancy along with ultrasound measurements for Crown-rump length (CRL), head diameter (HD), and gestation sac diameter (GSD), as well as among days for pregnancy with macroscopic measurements for CRL(US, mm) was shown to be positive and significant is in (Figure 2).

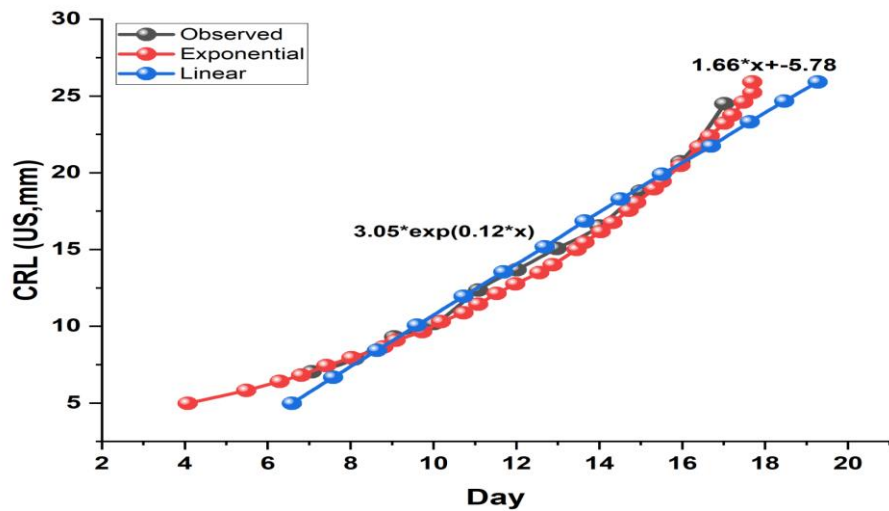


Figure (2): The link between days of pregnancy using ultrasonographic and macroscopic based on CRL

Additionally, there was a significant association between the macroscopic measurement of CRL and HD and ultrasonography. Ultrasonography may determine pregnancy in golden hamsters by detecting the presence of gestational sacs or embryos in the uterus. Observation by sight is one of the easiest techniques to detect pregnancy in golden hamsters. Pregnant female hamsters will show morphological changes such as an expanded belly and prominent mammary glands.

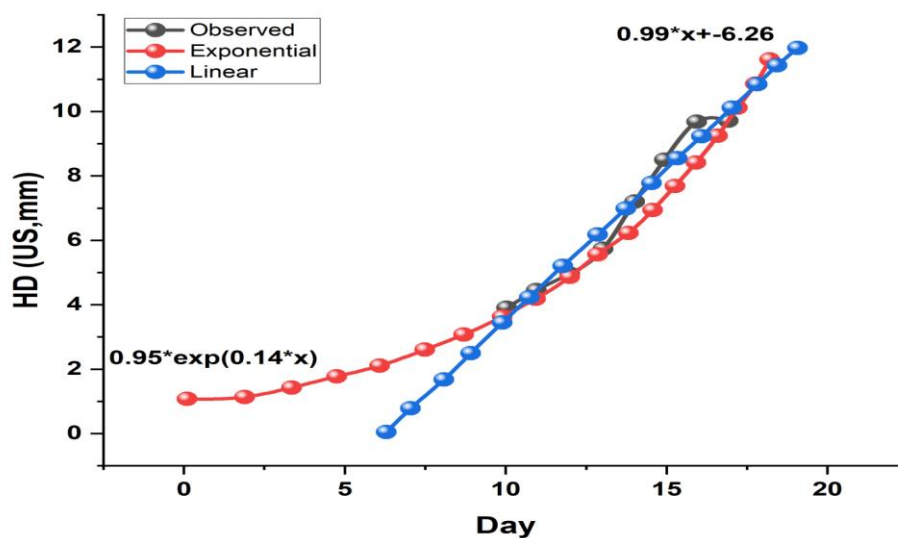


Figure (3): Correlation between days of pregnancy using US and MAC based on HD

This approach enables the diagnosis of pregnancy as early as 7-10 days after mating. In (Figure 3) the relationship among days of pregnancy using ultrasonographic and macroscopic based on HD (US, mm). It is a non-invasive technique that enables real-time imaging and is

usually thought to be safe for both the mother and the growing embryos, (Figure 4) shows the correlation between days of pregnancy using US and MAC based on crown-rump length (Mac, mm).

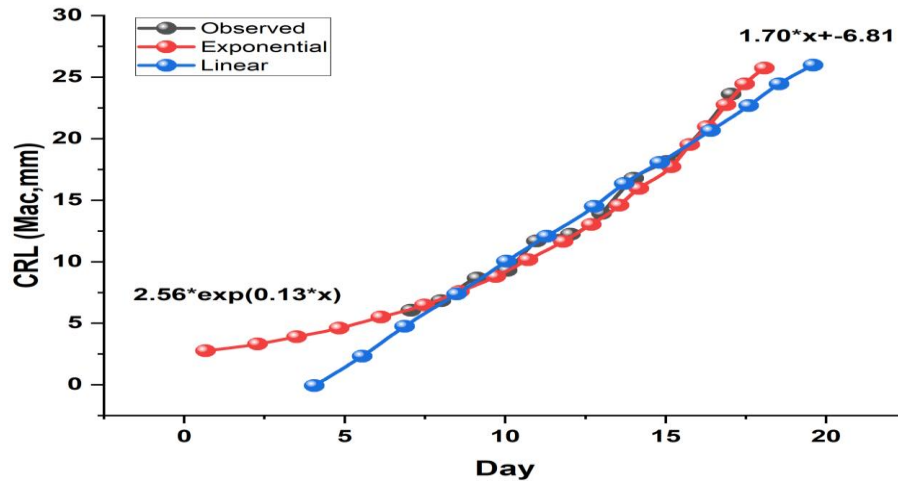


Figure (4): Correlation between days of pregnancy using US and MAC based on CRL (Mac,mm)

The macroscopic technique entails physically inspecting the animal's belly and reproductive tract for evidence of pregnancy. To examine changes in the reproductive organs, it depends on external observations and physical palpation. There was also a substantial correlation between the macroscopic assessment of GSD and ultrasonography. Ultrasonography may identify the existence of gestational sacs or embryos in the uterus and hence determine pregnancy in golden hamsters.

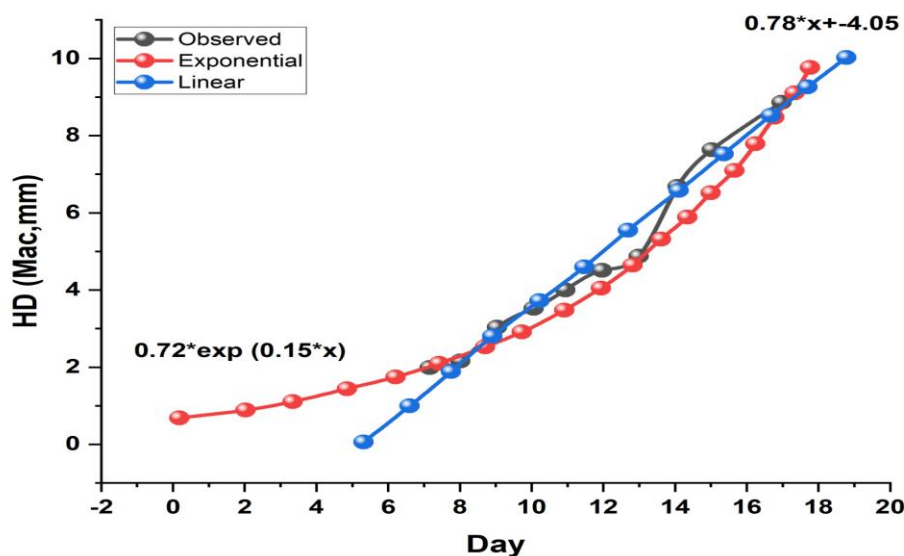


Figure (5): The correlation between days of pregnancy using US and MAC measures of a pregnant based on HD (Mac, mm)

The use of ultrasonic waves to see the inside architecture of the body is known as ultrasonography. It has the potential to be a non-invasive and reliable approach for detecting pregnancy in golden hamsters. In (Figure 5) the relationship among days of pregnancy using US and MAC measures of a pregnant is displayed.

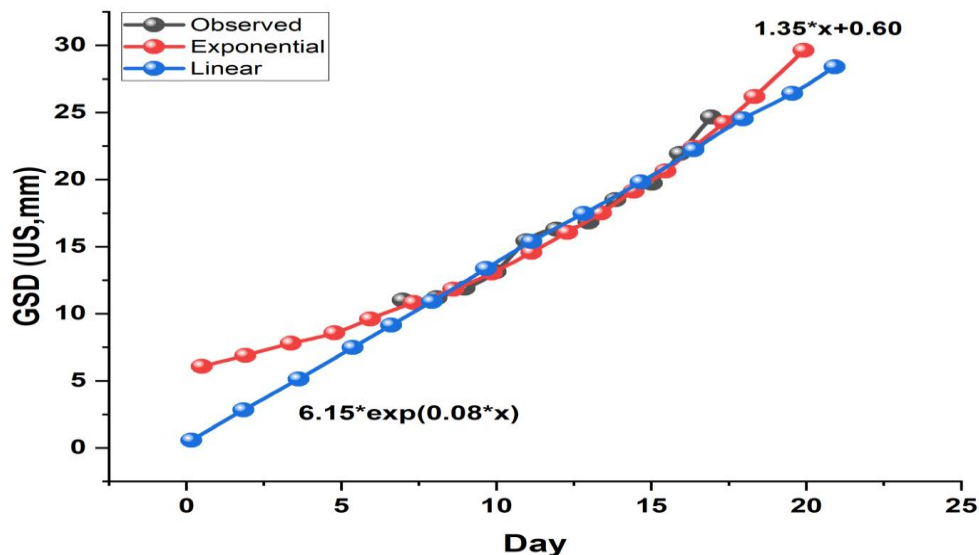


Figure (6): A link between days of pregnancy and US and MAC parameters was found in a pregnant golden hamster's fetus and GSD

Ultrasonographic examination provides for the observation of growing embryos or fetuses in the uterus, allowing for precise pregnancy status assessment and litter size prediction. In (Figure 6) and (Table 1) pregnant golden hamster's fetus and gestation sac, a correlation between days of pregnancy and ultrasonographic (us) and macroscopic (mac) measures was provided. This approach is very effective for detecting early-stage pregnancy.

Discussion

In this study, trans-abdominal ultrasonography scanning allowed for the early pregnancy diagnosis in golden hamsters as early as day 7 of gestation. GSD, CRL, and HD are reliable indicators for determining the golden hamster's period of gestation. Abdominal palpation effectively detects Pregnancy in pregnant laboratory animals from their middle to late Pregnancy (24). For instance, the gestation period in rabbits is 35 days, along with day 12 of Pregnancy; abdominal palpation may be used to diagnose pregnancy. Ultrasound pregnancy diagnosis was far most precise than abdominal palpation. Additionally, when the abdomen is palpated, tumors and infections might be misinterpreted for pregnancy (25). Today, thanks to improvements in high-frequency ultrasound imaging, it was feasible to gather precise pictures for the internal organs of rodents like mice and rats, including the heart and kidney. Laboratory animals' pregnancies have been examined with ultrasonography using linear

probes with a frequency range of 7.5–12 MHz (26). Research (27) using mice discovered that at higher frequencies, linear probes generated pictures with better resolution than curved probes. The current study's findings demonstrated that prenatal fetal ultrasonography assessments were often compatible with the macroscopic outcome, the macroscopic measures taken a right after the ultrasound test. It was recently suggested that golden hamsters' age after delivery might be predicted using ultrasound information from day 8 during pregnancy. During mouse research, using 10 MHz linear transducer, the embryo, and gestational sac should be identified by day 8. In second rat study (28), between 10 and 17 days post-coitum, trans-abdominal real-time ultrasonography with a 12-MHz linear transducer was used to examine the uteri for anesthetized Wistar rats. Using color and pulsed-wave Broadband ultrasound, the embryonic heart rate was calculated. The detection of the embryonic vesicles had a false-negative diagnostic rate of 25% on day 9, 8% on day 10, and 0% on subsequent days. Heart rate and crown-rump length were measured on day 15. By day 9 post-coitum, hamsters showed ultrasound signs of pregnancy. The pregnancy diagnosis had been confirmed by day 14, while the embryonic heartbeat was discovered. Between days 9 and 16, embryonic features might be seen using ultrasound. This research discovered that the ultrasonic diagnostic values were less than macroscopic. The fetus' curved and unique form within the gestational sac may be to blame for this disparity in results. However, it seems clear that during pregnancy, the typical embryonic vesicle's top diameter and size, together with crown-rump length, all increase along with the fetus's growth. The current research found that the fetal growth rate rose higher toward the end of pregnancy than it did throughout the beginning and middle of it. The average CRL size was 11.071.05 mm for the first three days, 15.61.4 mm in the second three days, and 20.890.79 mm in the third days (Table 1) thought the pregnancy period until day 9 was broken down into three periods of around three days each. The results demonstrate that the fetus developed much faster in the latter trimester. Thus, it may be inferred that fetal development in golden hamsters was quicker in the last stages of pregnancy. Numerous types of research have been done on the impact of cancer or drug usage while pregnant. Congenital cardiac disease was the most common type of non-infectious effect of infant death (29). Modern techniques like ultrasound imaging enable real-time visualization of defective hearts in heart-failure-affected embryos. These cutting-edge techniques provide us with useful information on the fetus' growth through illnesses. With the use of this data, researchers may look into any potential links between medications, poisons, and immunizations, the potential for miscarriage, and their effect on fetal growth throughout pregnancy on either hand along with another. The (30) viability of pregnancy and fetal development in various animals must thus be evaluated via further research.

Conclusion

In this work, transabdominal ultrasonography scanning in golden hamsters allowed early pregnancy identification as early as day 7 of gestation. It appears that GSD, CRL, and HD were reliable indicators to determining gestational age. The causes of ultrasonography on the development for embryonic internal organs should be a subject of future research, it is

recommended. To better understand pregnancy, hereditary illnesses, and the effects of medications on the baby, it would be helpful to do studies on how each organ develops throughout pregnancy. Prenatal biometric measures to determine fetuses' weight are also advocated, including biparietal diameter and fetal abdominal circumference. There are several restrictions to take into account when assessing the effectiveness of Ultrasonographic along with macroscopic techniques for pregnancy diagnosis in golden hamsters. The ultrasound technology with improved resolution and sensitivity might allow for more precise and detailed imaging of Golden Hamster reproductive organs as well as growing fetuses. This would allow for improved imaging of early-stage pregnancies and identification of minor pregnancy changes were enhanced in future.

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