

Ultrasonografical Monitoring as Diagnostic Tool for Reproductive Management in Female Buffaloes (*Bubalus bubalis*)

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Abstract

The aim of this study was to estimate the frequency of physiological and pathological reproductive conditions in female Italian Mediterranean Buffaloes. Female animals (n=444), ranging from 3 to 14 years of age were monitored by ultrasonography (USG). A total of 7319 USG images were collected and analyzed for pregnancy status, fetal sexing, gynecological measurements and pathologies. While pregnancy rate was determined as 38.75% and ovarium-genital canal disorders as 11.71%; the most common pathologies in nonpregnant animals are defined as ovarian cystic degeneration (OCD), hydrosalpinx and salpingitis (SALP), metritis, pyometra (METR) and pneumovagina (PVAG). OCD represented 4.27% of pathological conditions during long day season (February-June) with a higher frequency above 5 years of age (P<0.05); and characterized by the presence of rounded anechoic structures with a 3.12±0.54 cm outer and 2.5±0.22 cm an internal diameter. While SALP frequency was determined as 6.75% and more intense over 7 years; METR and PVAG frequencies were 0.67% in total. In conclusion, these results demonstrated that ultrasonographic monitoring is a useful diagnostic tool to optimize the reproductive management through pregnancy and delivery time determination in addition to certain pathological conditions of the reproductive tract in buffaloes.

Keywords: Buffalo, Reproductive pathologies, Reproductive management, Ultrasonography

Dişi Mandalarda (*Bubalus bubalis*) Reprodüksiyon Yönetiminde Tanı Yöntemi Olarak Ultrasonografik Görüntüleme

Öz

Bu çalışmanın amacı, dişi İtalyan Akdeniz Mandalarında fizyolojik ve patolojik reproduktif durumların frekansının belirlenmesidir. 3-14 yaş aralığındaki dişi hayvanlar (n=444) ultrasonografi (USG) ile görüntülenerek, toplam 7319 USG görüntüsü ile gebelik durumu, fetal cinsiyet, jinekolojik ölçümler ve patolojiler açısından analiz edildi. Gebelik oranı %38.75 ve ovaryum-genital kanal bozuklukları %11.71 olarak belirlenirken; gebe olmayan hayvanlarda en sık görülen patolojiler ovaryumun kistik dejenerasyonu (OKD), hidrosalpinx ve salpingitis (SALP), metritis, pyometra (METR) ve pnömovagina (PVAG) olarak tanımlanmıştır. OKD, uzun gün mevsimi süresince (Şubat-Haziran) ve 5 yaş üstü dişilerde (P<0.05) patolojik koşulların %4.27'sini temsil ederken; ortalama 3.12±0.54 cm dış ve 2.5±0.22 cm iç çapa sahip yuvarlak anekoik yapıların varlığı ile karakterizydi. SALP sıklığı %6.75 ve 7 yaş üzeri mandalarda daha yoğun tespit edilirken; METR ve PVAG frekansları toplamda %0.67 olarak belirlenmiştir. Sonuç olarak, reproduktif kanalın bazı patolojik koşullarının belirlenmesine ek olarak gebelik ve doğum tarihinin ultrasonografik görüntüleme ile saptanmasının mandalarda reproduktif bir yönetim aracı olarak kullanılabilirliği ortaya konulmuştur.

Anahtar sözcükler: Manda, Reprodüktif patoloji, Reprodüksiyon yönetimi, Ultrasonografi

INTRODUCTION

Ultrasonography (USG) monitoring holds a great potential for the reproductive management of dairy buffalo industry. Initially, it was an "elite" technique that was used only in a

limited way by some veterinarians and solely in horse and cattle rearing^[1-3]. Nowadays, USG is a widely used technique not only in research but also in the management of the reproduction in farm animals. As for Buffalo, as well as on cattle, it was used to diagnose pregnancy and detection



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of some pathological conditions in reproductive tract and ovaries, but now the use is considered as a diagnostic method to evaluate the reproductive performance, as well as a method of support to plan and to implement assisted reproduction techniques and farm management [4,5]. USG of the genital tract and ovaries allows to perform artificial insemination on more accurate time, pregnancy diagnosis following the implementation of the Ov-synch protocol in postpartum in order to implement the re-synchronization of those non-pregnant buffaloes. This protocol also allows to synchronize and inseminate a group of buffaloes without identifying the signs of estrus and to manage the postpartum period. USG monitoring allows identifying the pregnant and non-pregnant buffaloes through the present appearance of follicles and corpora lutea after insemination not only in an early manner, also simultaneously creates an opportunity for a management tool. Indeed, USG system is very useful for pregnancy diagnosis, since it allows performing diagnosis of pregnancy around 26-27 d, approximately 7 d anticipating the diagnosis by rectal palpation. The pregnancy diagnosis by rectal palpation, in fact, has an accuracy of 60% when performed between 31 and 40 d from the coupling, 92% when performed between 41 and 50 d and 100% between 51 and 55 d [6]. Recently some studies performed to demonstrate that corpus luteum (CL) can be considered to distinguish between pregnant and non-pregnant buffaloes [7,8]. These studies have shown, between 5 and 10 d after artificial insemination, an increase in the area and the diameter of CL can be detected in pregnant buffaloes, while not evident in those where conception has failed. CL functionality is also crucial in the early days of pregnancy which can be assessed by color Doppler and shows off the blood flow to his load. In fact, this flow tends to be higher in pregnant buffaloes 18 d after the insemination, compared to those which do not become pregnant. Reproductive evaluation also allows programming the additional synchronization (Re-synch) of the non-pregnant animals as soon as possible, where there is evidence for more than a centimeter-sized follicle, in the presence of a CL in regression. Studies in this regard have shown that there are no significant differences in conception rates according to the period of post-partum. Although the ideal to get a 12-month calving interval should be inseminating the buffaloes within 60 d after delivery [9]. In this regard, it can be suggested that real-time US monitoring is essential as a reproductive management tool to decrease the service period.

In buffalo breeding, anestrus (absence of estrus signs) and prolonged postpartum acyclicity (absence of ovarian cyclic activity) are the main reasons of economic loss. In dairy buffaloes first ovulation as detected by progesterone analysis and rectal palpation occurred between 24-55 d and 28-71, respectively, after calving and postpartum estrus occurred between 44 and 87 d [10]. In abattoir studies, oviductal pathologies have been largely mentioned in

buffalo and include pyosalpinx (0.6%-11.9%), hydrosalpinx (0.7%-14.2%), salpingitis (0.2%-14.2%), blockage of the oviduct (1.2%-37.8%) congenital defects (0.2%) and adhesions (1.5%-1.7%) [11]. Apart from above mentioned problems, fimbriae cover a greater area than in the bovine, which may give rise to adhesions causing the onset of acute salpingitis with consequent hydrosalpinx in Buffalo. However, a linear probe with frequencies higher than 7.5 MHz presents a good resolution and allows to evaluate anatomical structures as well as detecting the presence of follicles and CL, pregnancy diagnosis and to perform fetal sexing.

Through the evaluation of the pregnancy, fetal measurements, vitality, and pathological conditions of the reproductive tract with the US reproductive efficiency improves thus the interval between artificial insemination and service period decreases. Therefore, we aimed to report USG findings in the reproductive tract and ovaries of female buffaloes during long term monitoring activity and concurrently to define the frequency of physiological and pathological condition diagnosed with USG in a buffalo farm during reproduction management.

MATERIAL and METHODS

Ultrasonography monitoring was carried out with 444 buffaloes in a single farm, located in the northeast Italian territory (Venice) raising mainly Italian Mediterranean Buffalo. The US, with adjoining image collection, has been done in the period between February of 2013 and July of 2015 during the breeding season. The USG examination carried out transrectally with linear array probe (10 MHz) (MyLab Vet-One, Esaote, Italy) within the scope of the entire reproductive system; vagina, through the scanning of a body, both uterine horns up to the ovaries respectively. This study was approved by the Animal Care Ethics Committee of Ankara University and all experiments were conducted according to ethical principles and laws (2013-15-114).

Ovarian and Uterine Ultrasonography and Image Evaluation

The US images were classified considering the structures during the monitoring (gestational sac, vaginal-uterine and salpinx pathological conditions, follicles, CL and OCD) and defined remotely. OCD was characterized by the presence of rounded anechoic structures bigger than 2.7 cm and in absence of a functional CL [12]. OCD measurements considered: total diameter of the cyst; diameter of the internal cavity; thickness of the cyst wall. A follicular cyst is a structure which characterized by spherical shape; with a thin wall and regular border, less than 3.5 mm thickness; cavities homogeneously anechoic. The distinction of the luteal cyst from the follicular cyst is based on the wall thickness greater than 3 mm; irregular border; internal cavity with inhomogeneous content [13].

In fact, characteristic of the luteal cyst is the ability to see, inside the cavity of the cyst of the hyper-echogenic septa [14]. SALP condition was characterized by the presence of abundant anechoic areas within the oviduct. METR is determined according to the presence of anechoic fluid in cases of endometritis along with snowy echogenic particles [15]. PVAG is characterized by the presence of anechoic air within the vagina or both vagina and uterus [16]. Dimensional image measurements were done with ImageJ software.

Ultrasonography in Pregnancy

Ultrasound image classification was carried out according to Pieterse et al. [17], Pawshe and Purohit [18] (Table 1).

Within the same scope, determination of gestational age and predicted delivery time, embryo-fetus measurements were made and following fetal parameters [19]: Crown-rump length (CRL), biparietal diameter (BPD) and placentome diameter (PLD) were recorded. Fetal sexing (FS) were performed considering the genital tubercle (lobed structure, two hyper-echogenic parallel lines) close to the umbilical cord or close to the tail base. As a further aid, FS was examined between the hind legs to the scrotum or bladder [19]. Male FS was recorded when the genital tubercle was identified caudal to the point of abdominal insertion of the umbilical cord or detected in between the hind legs of the scrotum. Female FS was recorded when genital

tubercle was identified close to the tail base or rudimentary teats [20,21].

Fetal Measurements and Gestational Age Estimation with Ultrasonography

Measurements regarding the fetal parameters were then inserted into the equations identified by the Ali and Fahmy [19] expressing the relationship between the different fetal parameters. The fetal parameters were expressed in centimeters and the gestational age was calculated in weeks.

The equations used are: $y = 0.0282x^2 + 0.1589x - 0.1427$ where $y = \text{CRL, cm}$; $x = \text{Gestational Age, weeks}$ ($R^2 = 0.9451$); $y = 0.0179x^2 - 0.1222x + 1.0688$ where $y = \text{BPD, cm}$; $x = \text{Gestational Age, weeks}$ ($R^2 = 0.8999$); $y = -0.0031x^2 + 0.2712x - 0.9265$ where $y = \text{PLD, cm}$; $x = \text{Gestational Age, weeks}$ ($R^2 = 0.6792$). Gestational age of the fetus was calculated through these equations, which were expressed in weeks. They were then calculated the missing weeks at the end of gestation whereas the duration of pregnancy in Italian Mediterranean Buffalo is 315 d, which corresponds to 45 weeks. Thus, the obtained results and FS were compared with the actual dates of birth and sex of the calf.

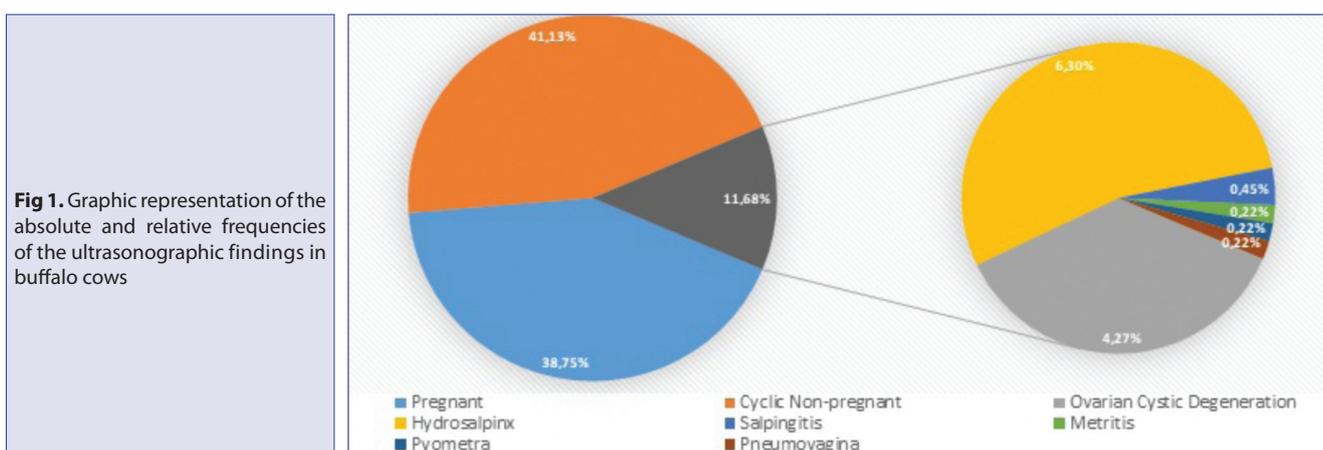
Data Analysis

On-farm and remotely collected data were analyzed using Excel datasheet and Image-J. The frequencies of parameters were calculated. Significant statistical differences between a month of monitoring in terms of frequency and estimated (embryo-fetal measurements) and actual delivery time were tested using Sigmapstat 2.03.

RESULTS

Four hundred and forty-four buffalos were examined by a total of 7319 US images. According to results with USG, 38.75% of buffalos were detected as pregnant and, 11.71% of animals had genital tract disorders while the rest of the animals were identified as cyclic, non-pregnant animals (Fig. 1).

Day	Structure	Reference
19 th	Embryonic vesicles	Pieterse et al., 1990 [17]
40 th	Umbilical cord	Pawshe and Purohit, 2013 [18]
42 th	Head	Pawshe and Purohit, 2013 [18]
44 th	Trunk and limb buds	Pawshe and Purohit, 2013 [18]
50 th	Cotyledons	Pawshe and Purohit, 2013 [18]
55 th	Fetal ribs	Pawshe and Purohit, 2013 [18]
62 th	Brain ventricles eye patch, the front and rear paws	Pawshe and Purohit, 2013 [18]



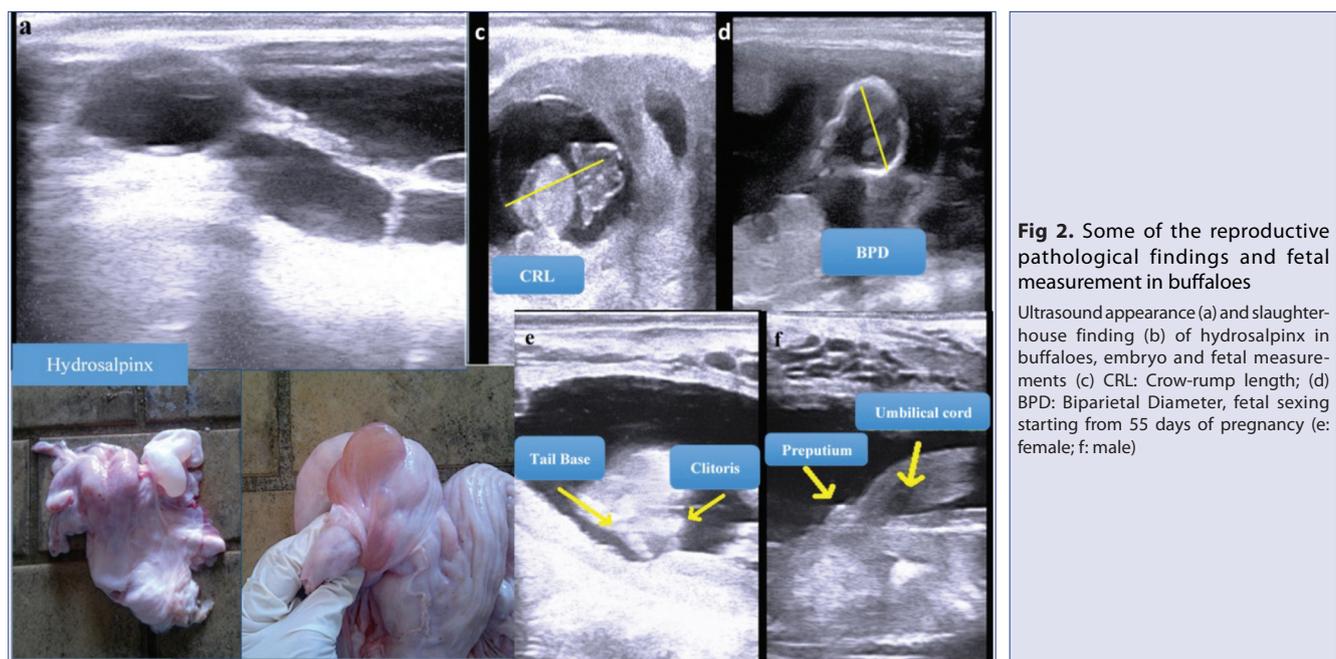


Fig 2. Some of the reproductive pathological findings and fetal measurement in buffaloes

Ultrasound appearance (a) and slaughterhouse finding (b) of hydrosalpinx in buffaloes, embryo and fetal measurements (c) CRL: Crow-rump length; (d) BPD: Biparietal Diameter, fetal sexing starting from 55 days of pregnancy (e: female; f: male)

It was possible to identify the presence of the fetus on images of 66 buffaloes, and the measurements of fetal parameters in 46 buffaloes for a total of 49 parameters. These 14 are the measurement of the CRL, 15 of BPD and 20 of PLD. Fetal measurements were calculated to estimate the delivery time equations reported by Ali and Fahmy^[19]. It was possible to compare the estimated delivery date with the actual one and were calculated the differences in between. CRL gives an estimation with an error varying from 2 to 12 d; BPD gives a difference from 2 to 14 d, while in the case of PLD varies between 14 and 99 d. In 66 buffaloes in which is highlighted by the presence of the fetus, the fetal sexing was performed in 9 animals. USG fetal sexing was possible to compare with the gender of neonates (n=5) resulting in a 4 of 5 right determinations. It should also be noted that fetal sexing, comparing the date of execution of USG and the birth date, was performed between 8 and 15 weeks of gestation.

The evaluation of ultrasound images made it possible to carry out the diagnosis of female genital tract disorders in 11.71% of the buffaloes. Diseases identified as: ovarian cystic degeneration (4.27%), hydrosalpinx (6.30%), salpingitis (0.45%), metritis (0.22%), pyometra (0.22%) and pneumovagina (0.22%) (Fig. 2). The cystic degeneration diagnosis was made on 19 subjects of total herd. The total measurements made highlights such as the cystic structures shown in the ultrasound image level on average, a total diameter of 3.29 ± 0.54 cm, a diameter of the internal cavity of 2.56 ± 0.22 cm and a wall thickness equal to 0.45 ± 0.14 cm. Cysts were found mainly in the period between February and June and in subjects over the age of five years ($P < 0.05$). The diseases diagnosed other than cystic degeneration have been reported in subjects aged between 4 and 14 years, with a higher incidence in those with more than 7 years.

Ultrasound appearance (a) and slaughterhouse finding (b) of hydrosalpinx in buffaloes, embryo and fetal measurements (c) CRL: Crow-rump length; (d) BPD: Biparietal Diameter, fetal sexing starting from 55 days of pregnancy (e: female; f: male)

DISCUSSION

This study presented the results from the ultrasonographical monitoring of the buffaloes' genital tract and ovaries. It has been shown that the ultrasound monitoring has enabled the assessment of the state of the ovaries, making a diagnosis of pregnancy and to detect the presence of reproductive anomalies.

In buffalo reproduction management, natural service is still widespread due to the certain seasonality, expression of estrus sign, high variability of ovulation time and thus reduced reproductive efficiency. This means that the exact date of fertilization generally is not known. However, owing to USG, it is possible to estimate the gestational age and the birth date by measuring these fetal parameters. The substitution in this equations according to Ali and Fahmy^[19] shows that fetal parameters that allow a more accurate estimate of the date of birth are the CRL and BPD, considering as acceptable with a maximum of 25 d difference between the estimated date and the actual date of birth. However, Placentomes diameter (PLD) is found to be an unreliable parameter for estimating the date of confinement^[19]. This outcome is also in line with the present study as PLD gave an estimation with an error varying from 14 to 99 d.

During the estimation of the delivery date, it was also possible to sex the fetus with an accuracy of 80%. This result

can be considered admissible comparing to the accuracy deviates highlighted in the study by Ali and Fahmy^[19], equal to 97.1%, (in general the accuracy of fetal sexing by ultrasound examination is between 95% and 100%). Our lower accuracy can be related to the fact that the number of fetal sexing performed in this case is reduced, in addition to the fact that a more accurate diagnosis for a single image, as has occurred in some of these cases, the abdomen of the fetus in level of the umbilical cord or of the area between the limbs is not sufficient. However, it would take the more accurate diagnosis of the images continuously at the passage of the probe on the ventral abdomen, the umbilical cord to the tail of the fetus. The fetal sexing was performed between 8 and 15 weeks of gestation, a result that is consistent with that observed in the study by Ali and Fahmy^[19], in which sexing was possible from the eighth week of pregnancy. Thus, compared to the number of pregnant buffaloes that were detected with the presence of the fetus, the low number of fetal parameters measurements and fetal sexing can be linked to the gestational age, in which the diagnosis has been made, considering that the genital tubercle begins to migrate from the 45th d of intrauterine life and only reaches the final position starting from the 55th d of pregnancy.

The ultrasound examinations and the remote image analysis has also allowed the detection of genital tract diseases. Consequently, the reproductive tract disorders are covering a considerable percentage of the problems in buffalo breeding. In fact, these problems result in economic losses due to reduced fertility with a calving interval extension, treatment costs and loss in milk production. With an early detection of the problem enables early intervention thus managing to minimize the economic losses. Ultrasound visits over the years on the farm and the subsequent evaluation of images showed the most frequent diseases were ovarian cystic degeneration (4.27%) and hydrosalpinx (6.30%). The incidence of ovarian cystic degeneration in buffaloes is very low; in clinical trials, it is variable from 0.07%^[22] to 1.48%^[23]. Several studies have been made against ovarian disease in buffaloes, and specifically of cystic degeneration, but a perfect clinical description of the latter, in buffaloes, does not yet exist. In fact, it can be said that there is a lack of information about biometrics of ovarian cysts in buffaloes as well as limited information on the physical, biochemical, hormonal and mineral composition of this disease^[24]. Aiumlamai et al.^[25] argue that ovarian cyst, in buffaloes, presenting as a follicular structure with a diameter greater than 2.5 cm. Other authors^[26] argue that ovarian cysts are always anovulatory structures with a diameter larger than 2 cm and which, persist for a variable period in the absence of the CL. The use of ultrasound for the diagnosis of cystic degeneration in buffaloes turns out to be an advantage since it allows to highlight, with greater accuracy and speed to assess the size of the cyst allows evaluating the absence of the CL, which is a necessity to classify ovarian

cystic degeneration. Relying solely on behavioral changes linked to ovarian cystic degeneration and rectal palpation is not ideal in buffaloes since the behavioral alterations in buffalo, unlike the bovine, are not very noticeable. Rectal palpation can be run into an incorrect diagnosis, as in buffalo ovarian structures appear to be located deeper in the ovarian stroma. It is possible to detect the presence of cysts in an early manner, i.e. before the onset of symptoms via ultrasound, thus allowing timely intervention on the problem for reducing the calving interval in buffaloes. Generally, although the incidence of this condition is not very high, the early diagnoses allow the breeder to solve the problem where the buffaloes are reduced their reproductive efficiency with cystic degeneration more than cows^[27].

The identification of such problems like hydrosalpinx, salpingitis and pyometra in the reproductive tract is very important since it affects fertility and reproduction and, consequently, reproductive efficiency. A load abnormality in any of the cervix, uterus or uterine horns can reduce the fertility in buffaloes^[28]. Studies carried out at the slaughterhouse showed an incidence of diseases borne by the oviduct between 10% and 29%^[29], with greater frequency of salpingitis. However, the hydrosalpinx compared to salpingitis are more frequent. Similarly, in the present study, the frequency of hydrosalpinx was 6.30% whereas salpingitis frequency was 0.45%. Hydrosalpinx is ultrasonographically notable for the presence of small anechoic areas, dates from the accumulation of fluid in the oviductal lumen, up to the complete relaxation of the oviductal wall in severe cases. It is manifested through the amber fluid accumulation, resulting in oviductal distension based on the severity of the injury. In this study, the percentage of buffaloes suffering from hydrosalpinx is found to be in line with the variable incidence between 0.7% and 14.3% reported in other studies^[30,31]. Consequently, an early identification of pathological conditions of the oviduct is essential for conception and embryo survival in the days before the descent into the uterus^[28]. These pathologies can lead to infertility and sterility in buffaloes, where the obstruction of the oviductal lumen prevent the encounter of gametes or by creating an environment unsuitable for embryonic development. Thus, it can be clearly suggested that USG is essential for the diagnosis of hydrosalpinx where there are not noticeable clinical symptoms or behavioral changes comparing to cystic degeneration.

In conclusion, this study has shown that the use of ultrasound can be used for different purposes in order to optimize the management of the reproduction consequently with pregnancy diagnosis. Primarily, the implementation of the re-synchronization program after insemination at postpartum where pregnancy fails. In the case of pregnant animals, the measurement of specific fetal parameters. Secondly, remote image analysis has allowed the detection

of genital tract diseases and an early identification of diseases to increase the reproductive efficiency. In conclusion, overall results show that ultrasound has a very important role in buffalo reproduction management.

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