Chapter 8

The Role of Trans-Rectal Ultrasonography in Artificial Insemination Program

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1. Introduction

The ultrasound technology has been used for various functions particularly as a tool in beef and dairy research for many years. It has become more available to the commercial livestock agriculture recently. Application of transrectal real-time ultrasonography in the study of bovine reproduction represents a technological breakthrough that has revolutionized the knowledge of reproductive biology [Boyd and Omran, 1991]. New research results derived from ultrasonic imaging has clarified the complex nature of reproductive processes such as ovarian follicular dynamics, corpus luteum function, and foetal development in farm animals. Extensive adoption and use of ultrasonography for routine reproductive examinations of dairy cattle is its highest contribution to the dairy industry.

Practical applications of ultrasound in bovine reproduction include imaging of the ovary as a diagnostic aid, examination and confirmation of ovarian cysts, early pregnancy detection, identification of twins and foetal sexing [Fortune et al, 1988; Garcia et al, 1999; Noble et al, 2000 Lamb, 2001; Stroud, 2006]. Although rectal palpation is the established method for conducting reproductive examinations, the information-gathering capabilities of ultrasonic imaging far exceed those of rectal palpation. Assessment of pregnancy status and foetal viability early post breeding can significantly improve reproductive efficiency. Such methods can play a key role in reproductive management to rapidly return open animals to the breeding program. Early pregnancy detection is only useful when techniques have a high level of accuracy for detection of both pregnant and non-pregnant animals.

Early ultrasonic identification of twinning or male calves in dairy cows allows for implementation of differential management strategies such as termination of the unwanted pregnancy or to mitigate the negative effects of twinning during the periparturient period. Ultrasound can accurately determine the presence of a viable embryo as early as 21 days after AI. The accuracy of detecting foetal viability may approach 100% [Lamb, 2001]. A technician with a trained eye has the capability of accurately assessing the age of the foetus based on foetal size [Curran, 1986]. At 60 to 85 days of pregnancy the trained user can even determine foetal sex by the absence and/or presence of the foetal genitalia with over 95% accuracy. These two features alone provide many options for the use of ultrasound in reproductive management practices [Palmer and Drinacourt, 1980; Muller and Wittkowski, 1986; Kastelic et al, 1989; Romano and Masgee, 2001]. Development of integrated reproductive management systems that combines ultrasound with new and existing reproductive technologies will further enhance the practical applications of ultrasonography. In summary, current and future applications of ultrasonography hold tremendous potential to enhance reproductive management and improve reproductive efficiency in bovine.

The incorporation of ultrasound in reproductive research has also led to greater understanding of ovarian physiology. Ultrasound has been used extensively in the development of controlled breeding programs involving both oestrus and ovulation synchronization for effective timed AI. Sequential monitoring of dynamic changes in a follicular population during the oestrous cycle has been made possible by ultrasonography [Driancort et al, 1991; Garcia et al, 1999; Ginther 1993]. This capability has helped unlock some of the mysteries of folliculogenesis. During anoestrous, inactive ovaries are readily differentiated from functional ovaries with ultrasonography.

Further, when choosing bulls, many producers are faced with difficult decisions regarding the contributions of both maternal and carcass traits. Not only do the attributes of multiple breeds vary, but variation within breed is also substantial. By combining ultrasound and AI, a producer can develop a breeding program that optimizes both maternal and carcass traits [Travene et al, 1985; Kahn, 1992]. Using ultrasound, producers may determine females that are pregnant with AI-sired heifer calves based on the age and sex of the foetus [Travene et al, 1985; Kahn, 1992]. In a typical commercial cow-calf production environment controlled breeding seasons range from 60 to 120 days. By using ultrasound as early as 30 days after the end of the breeding season in seasonally breeding animals, producers can divide their herd into cows that became pregnant early or late in the breeding season, and open cows which can subsequently be managed appropriately in accordance with their reproductive status to run the production at reasonable cost.

2. Evaluation of the ovaries during AI

Ultrasonic evaluation of the ovaries should be considered a routine part of the reproductive examination particularly in cattle. A 5MHz transducer has greater resolution and is more suitable for evaluation of the ovaries than a 3 or 3.5MHz transducer. Follicles, like other fluid structures, are non echogenic, and therefore, appear on the ultrasound image as black, circumscribed structures which are spherical to irregular in shape (Fig 1). The irregular shapes are attributable to compression by adjacent follicles and luteal structures of the ovarian stroma.

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Figure 1. Several medium sized follicles observed in mare. Ultrasonogram taken with 5MHz curvilinear array scanner (Hitachi 405, Germany). Source: Lemma et al, 2006

Follicles as small as 2 - 3 mm can be seen and the corpus luteum can usually be identified throughout its functional life [Lemma et al, 2006] Estimating the stage of oestrous cycle, assessing the status and number of preovulatory follicles, determining ovulation, monitoring the development and morphology of corpus luteum are among the potential applications of ultrasonographic examination of the ovaries [Fortune et al, 1988; Garcia et al, 1999; Noble et al, 2000; Evans et al, 2000; Evans, 2003]. The number and sizes of follicles on a given ovary will vary widely and are dependent on the time of year and the reproductive stage in different animals [Vandeplassche et al, 1981; Perry, 1991; Godoi et al, 2002]. Many small follicles are observed during early dieostrus and these follicles will grow larger at mid-cycle. The dominant or ovulatory follicle will develop at a rate of 1.5 to 2.5mm per day in cattle [Driancourt et al, 1988] few days before ovulation which are all easily monitored ultrasonically to determine the date of ovulation and hence subsequently fix the appropriate time for insemination. During oestrous cycles in cattle dominant follicles reach a maximum diameter of approximately 10 - 20 mm and the largest subordinate follicles reach maximum diameter of approximately 8 mm. Cows ovulate at about 12 hours after the end of the oestrus period. The time for insemination may therefore range between 6 and 24 hours prior to ovulation [Arthur, 2001; Ball and Peters, 2004]

The appearance of dominant follicles is often accompanied by an outward manifestation of behavioural oestrus. However, cows with dominant follicle that is about to ovulate do not always show overt oestrus and this is becoming one of the greatest hindrance to the success of AI. Whilst good oestrus detection does not necessarily guarantee good reproductive performance, poor oestrus detection makes poor performance hard to avoid [Arthur, 2001]. Poor oestrus manifestation and failure to detect oestrus further hinders insemination at the correct time which is an important cause of fertilization failure. Insemination very early in oestrus also causes reduced fertility; possibly due to reduced sperm survival rates before fertilization [Andrew *et al.,* 2004].

Many developing countries commonly relay on small holder dairy production system where owners cannot afford to keep a breeding bull and have to depend on AI services. The use of AI as the main method of breeding to improve performance means that the responsibility for oestrus detection falls upon the dairy owners who manage the herd [Lemma and Kebede, 2011]. In a study conducted to compare reproductive performance between farms using AI and natural service, the NSC, CCI and DALC were significantly higher ($p<0.05$) for farms using AI (2.1; 187.0 days and 185.7 days, respectively), compared to farms using natural service (1.7; 159.1 days and 154.9 days), respectively [Lemma and Kebede, 2011]. In a different study [Hansar et al, 2011] conducted to evaluate the efficiency of oestrus detection by small holder dairy owners in the success of AI and identify the role of ultrasonic evaluation of ovarian follicles before AI in improving pregnancy rate showed that ultrasonography can solve many problems of oestrus detection. In the same study, visual oestrus detection by dairy owners was assessed for validity using ultrasonic evaluation of the ovaries before AI with the following results (Table 1).

Oestrus signs	Overall (n=60)	Pregnant After AI (n=18)	Non-Pregnant After AI (n=42)
Bellowing [%]	60.0	61.1	59.5
Mounting others [%]	66.7	77.8	64.2
Vaginal discharge [%]	83.3	88.9	83.3
Mounting and vaginal discharge	60.6	87.5	52.0
Diameter of dominant follicle [mm]	12.9±3.4	14.7±3.3	12.1±3.37
Estrus to AI [hrs]	13.3±9.3	10.5±6.9	15.10

Table 1. Dairy cows and heifers that showed different types of oestrus signs prior to AI (n=60)

A significant difference ($p<0.05$) was observed in the mean diameter of largest follicle between the pregnant and non pregnant animals. Similarly, the duration from detection of estrus to AI was also significantly different ($P<0.05$) between successful and failed pregnancies. While none of the animals were waited until they exhibited standing estrus, the average duration to AI for animals that later became pregnant and showed both Vaginal discharge and mounting was 6.4hrs with 13.8mm average diameter of largest follicle. In contrast to this, those non pregnant animals showing both mounting and Vaginal discharge were brought to AI on average after 14.1hrs, and the average diameter of largest follicle was 9.7mm.

The mean (±SD) diameter of the largest follicle for animals showing both signs was 12.7±4.4 mm. Considering these estrus signs to be the best indicators of estrus as perceived by the dairy owners, 9, 5 and 6 animals were brought within 6 hrs, 7-14hrs and after 14hrs of heat manifestation, respectively. The mean size of the follicles for the same time group of animals was 13.5±3.2mm, 13.0±4.7mm and 11.1±5.8mm, respectively. Only 39.4% were found to be fit for insemination (showing most oestrus signs, follicular diameter ≥12mm and/or were brought for AI ≤12hrs of oestrus manifestation) during the ultrasonic evaluation. However, all animals were inseminated giving a pregnancy rate of only 30.0% compared to 61.5% for the fit animals alone. It was concluded that the low conception rate, delayed time of insemination, and the difference in the size of larger follicle indicate the incompatibility of visual heat detection and optimal time of insemination. This study clearly shows two important facts: cows showing oestrus do not necessarily carry mature follicles ready for ovulation, and ultrasonic evaluation of the ovaries right before AI can significantly improve pregnancy rate by avoiding insemination of animals that are not fit for service.

Furthermore, ultrasound has also great contribution to reproductive management through estrus synchronization. By early detection of pregnancy post AI in synchronization the pitfalls of different factors involved in reducing reproductive performance could be identified and accordingly mitigated. In a study [Hamid et al, 2012] conducted to evaluate the effects of artificial estrus induction and breed, both were known to have significant effect on pregnancy rate (Table 2). Pregnancy was determined at day 26 post AI using a B-mode real time ultrasound with a 5MHz linear array transducer (Mindray, Hong Kong). The negative effect of poor nutritional management appropriate for cross breed animals, and the positive influence of estrus induction on first service pregnancy rate were identified. This information was useful in the decision making process on those animals that failed to be pregnant.

Breed	Non oestrus induced cows			Oestrus induced cows		
	Nº of cows at first AI	Pregnancy rate [%]	p-value	Nº of cows at first AI	Pregnancy rate [%]	p-value
Zebu	75	18 (24%)		62	30 (48.4%)	
Cross	10	6 (60%)	0.018	15	7 (46.7%)	0.905
Total	85	24 (28.2%)		77	37 (48.1%)	0.009

Table 2. Comparison of first service pregnancy rate between oestrus induced and non induced cows (effect of heat induction); and between breeds (zebu and Holstein cross cows)

3. Application of ultrasonography in early PD

Reproductive ultrasonography has increased the knowledge of the changes during early pregnancy in different animals. With the use of a real-time, B-mode ultrasonography and 5 MHz transducer, pregnancy can be detected as early as 9 to 12 days post AI into gestation [Pierson and Ginther, 1984; Curran et al., 1986; Boyd et al., 1988]. A thorough understanding of the ultrasonic anatomy and dynamic changes in the uterus is essential for accurate pregnancy detection. The embryo initially appears as a short line (Day 20-22), later becomes C-shaped (Day 22-30), and finally, by Day 30-32 of gestation assumes an L- shape (Fig 2). Although the embryo can first be detected between Days 19 and 24 of gestation [Curran et al., 1986], it is most practical to scan females which are expected to have embryos that are >26 days of age. The bovine foetus can be visualized beginning at day 20 post breeding and continuing throughout gestation, however, because of its size in relation to the image field of view, the foetus cannot be fully imaged after 90 days using high frequency transducers.

Figure 2. Bovine embryo scanned on Day 26 of pregnancy using B-mode real time ultrasound with 5MHz linear array transducer

Accuracy of diagnoses improves, however, by Day 18 (85%), Day 20 (100%) and Day 22 (100%) of pregnancy (Kastelic et al., 1989). Presence and vitality of the embryo can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation. Early pregnancy detection has a greater economic significance particularly for the dairy industry. When an accuracy of over 99% is achieved, it enables to rapidly identify fertility problems. The rate of detection of early embryonic loss is thus also higher with 10 to 16% of cows diagnosed pregnant at 28 days post AI experience early embryonic loss by 56 days post AI. Cows diagnosed pregnant at 28 days post AI using ultrasound should, therefore, be scheduled for a subsequent examination around 60 days post AI, when the rate of embryonic loss per day decreases dramatically.

In situations where the exact dates of AI are known, ultrasonography is simply used as a confirmation of pregnancy or to validate that detection of a viable embryo within the first two weeks of pregnancy. In contrast, another study showed diagnosis of pregnancy in heifers on day 10 through day 16 of gestation resulted in a positive diagnosis for pregnant or non-pregnant cows in less than 50%. On from days 18 - 22 of gestation, accuracy of pregnancy diagnosis improved from 85% to 100% [Lamb and Fricke, 2005]. Although an embryonic vesicle is detectable by ultrasound as early as 9 days of gestation, accuracy of detection approaches 100% usually after day 25 of gestation. For practical purposes, the efficiency (speed and accuracy) of a correct diagnosis of pregnancy should be performed in cows expected to have embryos that are at least 26 days of age.

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Ultrasound is a rapid method for pregnancy diagnosis, and experienced palpators adapt to ultrasound quickly. This is particularly important to lessen the effect of early scanning on embryonic survival. The time required to assess pregnancy in beef heifers at the end of a 108-day breeding season averaged 11.3 seconds using palpation per rectum versus 16.1 seconds required to assess pregnancy and foetal age using ultrasound (Galland et al., 1994). Two points must be considered when using ultrasound for routine early pregnancy diagnosis in a cow herd. First, when using ultrasound for early pregnancy diagnosis, emphasis must be given to identifying non-pregnant rather than pregnant cows. Second, a management strategy must be implemented to return the non-pregnant cows to service as quickly as possible after pregnancy diagnosis. Such strategies include administration of PGF2α to cows with a responsive CL, use of estrus detection aids, or a combination of both methods. Such strategies will significantly improve outcomes of AI in large herds.

The main advantages of the use of ultrasound for pregnancy diagnosis are the high accuracy of the results and the relatively early confirmation of pregnancy. Mistakes can be made, no matter proficient the operator might be. Therefore, it is suggested that a female not be considered non-pregnant until two negative scans have been obtained at a gestation age of at least 16 days. The main disadvantages of the use of ultrasonography are related to the cost and time involved. Ultrasound machines are relatively expensive. Pregnancy diagnosis with an ultrasound machine takes more time than rectal palpation. Also the training required for proper interpretation of the images is another disadvantage. Cows diagnosed pregnant at an early ultrasound exam have a greater risk of early embryonic loss and, therefore, must undergo subsequent pregnancy examinations to identify and rebreed cows that experience such loss. If left unidentified, cows experiencing embryonic loss after an early pregnancy diagnosis would actually reduce reproductive efficiency by extending their calving interval (Fricke, 2002).

4. Early embryonic death (EED)

Prior to the development of ultrasound for pregnancy diagnosis in cattle, veterinarians were unable to accurately determine the viability or number of embryos or foetuses. Because the heartbeat of a foetus can be detected at approximately 22 days of age, one can accurately assess whether or not the pregnancy is viable. Studies in beef [Beal et al., 1992; Lamb et al., 1997] and dairy cattle [Smith and Stevenson, 1995; Vasconcelos et al., 1997; Szenci et al., 1998] have used ultrasound to assess the incidence of embryonic loss. The fertilization rate after AI in beef cows is about 90%, whereas embryonic survival rate is 93% by day 8 and only 56% by day 12 post AI [Diskin, and Sreenan, 1980]. The incidence of embryonic loss in beef cattle appears to be significantly less than in dairy cattle. Some reports [Beal et al., 1989; 1992; Lamb and Fricke, 2005] indicate a 6.5% incidence of embryonic loss in beef cows from day 25 to day 45 of gestation. In dairy cattle, pregnancy loss from 28 to 56 days after artificial insemination may reach 13.5%, or 0.5% per day. Ultrasonography hence becomes a useful tool in the study of early embryonic death (EED), and to monitor the success of a breeding program, by determining pregnancy rates and embryonic death.

Ultrasonography also provides a tool to accurately differentiate between the failure of a female to conceive and the incidence of embryonic mortality early after AI service [Beal et al., 1989]. At present, there is no practical way to reduce early embryonic loss in lactating dairy cows. However, recognizing the occurrence and magnitude of early embryonic loss may actually present management opportunities by taking advantage of new reproductive technologies that increase AI service rate in a dairy herd. If used routinely, transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by reducing the period from AI to pregnancy diagnosis to 26 to 28 days with a high degree of diagnostic accuracy [Beghelli et al, 1986; Cheffaux et al, 1986; Baxter and Ward, 1997; Meyers, 2002].

5. Conclusion

Currently fresh, chilled and frozen-thawed semen are used extensively for AI in animal breeding and production throughout the world. Dairy herd size has increased with increasing pressure to maximize milk yield, whilst at the same time reducing production costs is necessary. Accurate oestrus detection crucial for timed and successful AI and early detection of the success or otherwise of insemination can meaningfully reduce the time delay before repeated insemination if this is necessary. For these reasons it is very important to have a reliable method to accurately detect the occurrence of oestrus. Errors in efficiency and accuracy of heat detection result in high semen cost and an increase in the interval from calving to conception, reducing cow production and net returns. The use of ultrasonography prior to AI for determining the ovarian status can significantly improve the success of AI.

Conversely, reproductive management is currently a major factor affecting profitability in the dairy industry. Early identification of non-pregnant dairy cows and heifers post AI can improve reproductive efficiency and pregnancy rate by decreasing the interval between AI services and increasing AI service rate. Thus, imaging technologies such as ultrasound to identify non-pregnant dairy cows and heifers early after AI may play a key role in management strategies to improve reproductive efficiency and increase profitability on commercial and small holder dairy farms. Early detection of non-pregnancy will lead to earlier intervention and decision that will shorten the calving interval, which has economic effect on the diary farm. Transrectal ultrasonography has also been used to determine foetus viability and hence can improve the outcome of AI by allowing knowledge based decision on problem cows.

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