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Review paper

Non-invasive methods for diagnosing pregnancy in cows and their real value

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Abstract

Proper management of cattle reproduction has a major impact on the efficiency and profitability of dairy production. Ultrasound examination and transrectal palpation or the pregnancy-associated glycoprotein (PAG) test are currently the most commonly used methods for pregnancy diagnosis. However, alternative methods to those mentioned above are constantly being sought in order to minimise stress during the examination, the cost of veterinary services and to reduce the rate of errors in pregnancy diagnosis. Non-invasive methods of pregnancy diagnosis in cows are being improved, which include the barium chloride test, sulphuric acid, seed germination test, measurement of progesterone, interferon-tau or interferon-stimulated genes (ISGs), PAG, early pregnancy factor (EPF), estrone sulphate, thermography and electrocardiography. Over the past few decades, these methods have been extensively described. Some of these tests require blood, milk or urine for the diagnosis of pregnancy, while others require prolonged contact with the animal in order to take the appropriate measurements. Despite their advantages in terms of simplicity and lower cost compared with traditional methods of pregnancy diagnosis, they are sometimes problematic because of the difficulty of collecting material for testing. They allow the determination of a pregnancy without determining its age or pathology on the part of the development of the fetus and the reproductive system. They are also generally characterised by lower accuracy, sensitivity and specificity, which can have a negative impact on reproductive management and translate into the economics of dairy production. In the context of the above information, it appears that non-invasive methods of pregnancy diagnosis need to be further improved to minimise or eliminate the disadvantages cited.

Keywords: cows, non-invasive, pregnancy diagnosis

Introduction

The efficient management of cow reproduction is one of the important factors determining the profitability of dairy farms (Esslemont et al. 2001). Reproduction is influenced by, among others, metabolic and infectious diseases, adequate nutrition, housing conditions and herd management (Ferguson 1996, Santos et al. 2018). The goal of every dairy cow breeder is to achieve as short an inter-calving period as possible. To achieve this goal, early determination of pregnancy is necessary (Balhara et al. 2013). Currently, ultrasound, less commonly transrectal palpation, is used for this purpose. The use of ultrasound allows early diagnosis of pregnancy, determination of viability and sex of the foetus (Szenci et al. 1998). In addition to the numerous advantages, both methods have some disadvantages, some of which include: increased stress levels during and just after the examination (Nakao et al. 1994, Kovács et al. 2014), increased risk of embryo/fetal death after performed too early transrectal examination (Franco et al. 1987, Bond et al. 2019). Some alternatives to the cited methods for pregnancy diagnosis are non-invasive methods (Table 1). These are relatively cheap and simple and also in many cases can be performed on the farm (Haque et al. 2023). In addition, urine or milk can be used to perform such diagnoses, which guarantees easy sampling and preservation of cow welfare (Skálová et al. 2017, Lavon et al. 2022). However, their accuracy is not always satisfactory which, compared to classical pregnancy diagnostic methods, is a certain disadvantage (Rao and Veena 2009, Azmi et al. 2020).

Chemical methods

Barium chloride test

The first studies on the use of barium chloride in the diagnosis of pregnancy in cows appeared in 1966. Determination of pregnancy based on urine testing allows confirmation of pregnancy at 25-30 days post-fertilisation (Haque et al. 2023). A urine sample should be collected during spontaneous urination or catheterisation and then mixed in a 1:1 ratio with a 1% barium chloride solution. If progesterone is present in the urine (due to the presence of the corpus luteum during pregnancy), the solution remains clear, while turbidity of the sample indicates low concentrations of progesterone and thus no pregnancy (Lalrintluanga and Dutta 2009). The test has lower sensitivity, specificity and accuracy in detecting pregnant cows, compared to testing progesterone concentration in milk or blood, diagnosis of pregnancy *per rectum*, or ultrasound examination of pregnancy (Rao and Veena 2009, Azmi et al. 2020, Dana et al. 2020) (Table. 2).

Additionally, the test is not specific. It does not distinguish between ovarian cystic and dysfunctional corpus luteum compared to a normal gestational or cyclic corpus luteum. To improve specificity, urine protein levels can be determined in parallel to exclude ovarian cystic fibrosis, which is accompanied by an increase in protein levels above 100 mg/dl, while levels do not exceed 100 mg/dl in pregnancy (Haque et al. 2023). There are also some methodological inaccuracies regarding the time after which the test reading is taken, the volume of urine used for the test and barium chloride and its concentration.

Sulphuric acid test (H_2SO_4)

Concentrated sulphuric acid initially found application in the early diagnosis of pregnancy donkeys (Kubátová et al. 2016), and camels (Fedorova et al. 2015). The essence of the test is the reaction of sulphuric acid with oestradiol present in the urine of pregnant cows, resulting in fluorescence observed in the darkroom. An increase in estrogen secretion to determine estrogen concentration occurs around the 4th month of pregnancy. Normally oestrone (E1), oestradiol 17-α (E2-α) and oestradiol 17-β (E2-β) are marked, which are excreted, among other substances, in the urine (Veenhuizen et al. 1960). The test is performed by mixing 1 ml of urine with 5 ml of distilled water. The next step is to combine 1 ml of the resulting solution with 0.5 ml of 97% sulphuric acid (H_2SO_4). The use of such proportions allows the most reliable results to be obtained. In pregnant cows, the test shows pink fluorescence, while in non-pregnant cows fluorescence does not occur at all. Disadvantages of the test include its low accuracy (Table 3) and the need for proper storage and careful handling of concentrated sulphuric acid.

Biological methods

Arborisation/crystallisation test of cervical mucus or saliva

Crystallisation of cervical mucus or saliva is one of the biological methods of pregnancy diagnosis used in many animal species (Ježková et al. 2008, Pardo-Carmona et al. 2010). To perform this method, a microscope, a basic slide and the secretion to be examined are required. The amount of mucus and crystallisation patterns change due to hormonal changes during the sexual cycle (López-Gatius et al. 1993). The increase in cervical mucus crystallisation is dependent on the action of oestrogen, while this effect is reduced by the action of progesterone (Grant 1958). Both of these hormones act via steroid receptors on the mucus-producing cells, which alters its physicochemi-

Table 1. Classification of non-invasive methods of pregnancy diagnosis in cows

Chemical methods	Barium chloride test
	Sulphuric acid test
Biological methods	Arborisation/crystallisation test
	Seed germination/Punyakoti test
Hormonal and immunological methods	Estrone sulphate
	Pregnancy-associated glycoproteins (PAGs)
	Progesterone
	Early pregnancy factor (EPF)
	Interferon-tau (IFN-τ)
PCR methods	PCR interferon-stimulated genes (ISGs)
Other methods	Thermography
	Fetal electrocardiography

Table 2. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using barium chloride.

Autor	Days after artificial insemination (AI)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Pandit and Adhikari 2023	> 60	54.0	85.0	69.5
Dana et al. 2020	-	79.4	30.0	52.7
Azmi et al. 2020	-	100	13.21	28.13
Haque et al. 2023*	25-35	-	-	90.47
Haque et al. 2023*	45-55	-	-	50.0

* test performed with protein determination

Table 3. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using sulphuric acid.

Author	Days after AI	Sensitivity (%)	Specificity (%)	Accuracy (%)
Azmi et al. 2020	-	81.22	9.43	28.13
Setiawati et al. 2021	24	-	-	80.0
Setiawati et al. 2021	60	-	-	86.6

cal and structural properties. It is well known that during oestrus, coagulating mucus or saliva takes on a pattern reminiscent of a fern leaf (Hafez and Hafez 2000, Ježková et al. 2008). In addition, other crystallisation patterns can be distinguished (dotted, twiggy and their combinations, as well as atypical patterns). The best time to perform the test is between 20 and 29 days after insemination. This term is related to the subsequent ovulation occurring at that time. The mucus and saliva of cows that have not calved crystallise in the likeness of a fern leaf. A different situation exists in pregnant cows, in which the crystallisation pattern of cervical mucus or saliva may take on different patterns mentioned earlier. However, the implementation of this method requires samples to be collected over several days and consistently viewed under a microscope, which can be problematic with large numbers of animals (Skalova et al. 2013). On the other hand, according to other researchers, the saliva of all in-calf cows on day 16 after mating/insemination showed a twig fir type crystallisation pattern; however, a similar crystallisation pattern was recorded in 50% of non-calf cows. The authors also state that the test is more useful for determining the day of ovulation than confirming pregnancy (Noonan et al. 1975, Ježková et al. 2008). Besides the crystallisation pattern, the dry matter content of cervical and vaginal mucus should be also taken into account. It has been determined that the dry matter content of the mucus is lower during ovulation, when fern-leaf-like crystallisation is obtained, and similarly higher during the rest of the oestrous cycle, when crystallisation patterns are different. However, the method described lacks the required accuracy and strictly defined cut-off values that would clearly indicate the day of ovulation or calving (Noonan et al. 1975).

Seed germination/Punyakoti test

The origins of the use of the seed germination test for pregnancy diagnosis can be traced back to ancient Egypt - some 4,000 years ago (Rai et al. 2018). The test uses cow urine, diluted 1:4 with distilled water. About 15 ml of this mixture is placed on blotting paper with grains (wheat, maize, beans, etc.), placed in a sterile petri dish. After 3-5 days, the number of germinated seeds is determined, as well as the length of the growing shoots/sprouts, by relating the result of this trial to a control trial conducted on blotting paper soaked in distilled water (Narayana Swamy et al. 2010). In pregnant cows, the number of germinated seeds and the length of germinated sprouts is lower compared to seeds that were placed on the urine medium of non-pregnant cows. A stunting of seed growth or a shortening of the sprout/shoot length by 60% or more, compared to the number of germinated seeds or sprout/shoot length in the control sample, has been shown to indicate pregnancy. Conversely, a reduction in sprout/shoot length or inhibition of seed growth of less than 60% compared to the control sample indicates no pregnancy (Okunlola et al. 2019). This is due to the presence of abscisic acid, auxin, oestrogens and progesterone, among others, in the urine of pregnant cows, which inhibit seed germination (Veena and Narendranath 1993). This method can be used successfully from the 2nd month of pregnancy (Rine et al. 2014). Similar to the barium chloride test, the results obtained differ in accuracy from those obtained by *per rectum* palpation or ultrasound. In one study (Hussain et al. 2016) pregnancy was confirmed using this method in 92.3% of cows that had previously been determined to be in calf by *per rectum* examination. In contrast, other researchers (Azmi et al. 2020) after validation using urine from pregnant and non-calf cows, obtained an accuracy of 74.55%, with a sensitivity and specificity of 45.46% and 81.82%, respectively.

Hormonal and immunological methods

Estrone sulphate concentration

Non-invasive methods of pregnancy diagnosis also include the measurement of estrone sulphate concentration which is the main placental estrogen synthesised by the cotyledons of the placenta of cows (Hoffman et al. 1979, Robertson and King 1979). Its levels can be determined in milk (Prakash and Madan 1993), urine (Yang et al. 2003), feces (Hoffmann et al., 1997) and blood (immunoenzymatic methods) (Henderson et al. 1994). An increase sufficiently significant to allow diagnosis of pregnancy does not occur until 14-17 weeks of gestation (Hamon et al. 1981). The concentration of oestrone sulphate in the blood depends, for instance, on breed, maternal weight and fetal sex (Lobago et al. 2009) and increases day by day until the end of pregnancy (Shah et al. 2006). In addition to domesticated animals, it can also be detected in the feces of wild or zoo animals (Chapeau et al. 1993, Dufour et al. 2024). The measurement of estrone sulfate is applicable for example in Australia or New Zealand, where pregnancy in dairy and beef cattle is tested with the Confirm Plus test. Due to the late detection of estrone sulphate in blood, milk and urine, it is not suitable for the early diagnosis of pregnancy. However, it can be used to confirm later stages of pregnancy.

Pregnancy-associated glycoproteins (PAGs)

The first tests to detect pregnancy-associated glycoproteins (PAGs) were developed about 40 years ago (Sasser et al. 1986, Zoli et al. 1992). The test requires milk or serum. Pregnancy-associated glycoproteins present in the maternal circulation are derived from mononuclear and binuclear cells of the embryonic trophoblast. They are detected by ELISA using antibodies specific for PAG and horseradish peroxidase. The sample is then assessed for colour in a spectrophotometer to determine the concentration of PAG in the test solution. The optimal time to make a confident diagnosis is set at approximately day 32 after insemination (Ricci et al. 2015). The accuracy of the PAG ELISA test is similar to that of ultrasound diagnosis of pregnancy (93.7-97.8%) (Table 4). It is worth noting that primiparous cows have higher PAG levels in plasma and milk, in contrast to multiparous cows (Lobago et al. 2009, Ricci et al. 2015). In addition, PAG levels in milk and plasma of pregnant cows are negatively correlated with milk production, for both primiparous and multiparous cows (López-Gatius et al. 2007, Ricci et al. 2015). Plasma PAG concentrations are also influenced by breed, maternal weight, sex and birth weight of the calf (Lobago et al. 2009). The disadvantage of this method is the elevated levels of some types of PAG in the blood (pregnancy specific protein B - PSPB) between 80 and 100 days postpartum, which may translate into a misdiagnosis of pregnancy (Kiracofe et al. 1993, Kumar Bharti and Jacob 2019). Due to early embryonic mortality, all cows found to be in calf at the first test should be retested 70 days or later after artificial insemination (Ricci et al. 2015). Determination of PAG concentrations can also be useful in classifying a particular cow for a specific hormone program or in assessing the effectiveness of hormone treatment (Barbato et al. 2022, Thompson et al. 2010). Despite some drawbacks, the test is used successfully on industrial cattle farms in many countries around the world.

Table 4. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using PAG.

Author	Days after AI	Material tested	Sensitivity (%)	Specificity (%)	Accuracy (%)
Moussafir et al. 2018[1]	30-40	Blood	89.4	89.8	90.0
Moussafir et al. 2018[2]	30-40	Blood	100	81.3	94.0
Gajewski et al. 2014	35	Blood	100	100	100
Gajewski et al. 2014	35	Milk	97	100	100
Karakuş et al. 2020[3]	30	Blood	61.54	79.17	70.0
Karakuş et al. 2020[3]	30	Blood	50.0	75.0	62.0
Karakuş et al. 2020[3]	50	Blood	63.64	100	75.0
Karakuş et al. 2020[3]	50	Blood	50.0	100	65.62
Dalmaso de Melo et al. 2020[4]	25	Blood	98.0	86.0	92.0
Dalmaso de Melo et al. 2020[5]	25	Blood	99.0	87.0	94.0
Ghaidan et al. 2019	-	Blood	100	96.0	97.05
Roberts et al. 2015	37-125	Milk	99.7	80.8	-
Silva et al. 2007[6]	27	Blood	-	-	93.7

[1] – Ubio quickVET; BPRT®
[2] – Bovine Pregnancy Test DG29®
[3] – Fassisi® BoviPreg
[4] – heifers
[5] – cows
[6] – refers to first insemination

Progesterone determination

Progesterone concentrations in milk or plasma can also be determined to diagnose pregnancy in cows (Laing and Heap 1971, Shemesh et al. 1973). The progesterone-producing corpus luteum maintains the uterine endometrium in a state favourable to implantation and embryonic and fetal development (Anderson et al. 2008). The concentration of progesterone in milk or serum reaches a maximum value 13-14 days after oestrus and, if the animal is pregnant, remains elevated for longer, which is the basis for confirming pregnancy (Shemesh et al. 1973, Sasser and Ruder 1987). Concentrations >5 ng/ml in milk is sufficient to confirm pregnancy, while at values <2 ng/ml the cow is considered non-pregnant (Zaied et al. 1979). The highest rate of correct pregnancy diagnosis in milk is recorded between 24 to 27 days after insemination (Heap et al. 1976, Pope et al. 1976). Furthermore, higher concentrations of progesterone are recorded in milk from evening milking than from morning milking (Heap et al. 1976). Field tests, such as the P4 Rapid Test, can be used to make an early diagnosis of pregnancy based on the concentration of progesterone in milk (Bonev 2021, Mehmedi et al. 2021). Modern milking systems also make it possible to determine the concentration of progesterone in milk, which significantly facilitates reproductive management in the cattle herd (Blavy et al. 2018 Antanaitis et al. 2020). False-positive results are sometimes obtained with high progesterone concentrations in cases such as uterine infection, cystic ovaries or irregular estrous cycles (Kumar Bharti and Jacob 2019). The advantages of the progesterone determination include, as with other non-invasive methods of pregnancy diagnosis, the easy collection of the test material (milk) and the possibility of carrying out the test on the farm (Gowan et al. 1982, Pennington et al. 1985). The progesterone test has a higher pregnancy detection rate compared to another non-invasive method, the barium chloride test (Dana et al. 2020). Comparing the results of the *per rectum* palpation test and the progesterone assay for the diagnosis of pregnancy, similar accuracy was obtained, while the progesterone assay had a higher sensitivity than the *per rectum* palpation test (84.6%) (Table 5).

Early pregnancy factor (EPF)

Early pregnancy factor (EPF) has been identified in cattle, sheep (Nancarrow et al. 1981) and mice (Morton et al. 1987) using the rosette inhibition test (RIT). Early pregnancy factor is a substance with growth-regulating and immunomodulating properties that is required for successful pregnancy and proliferation of both normal and tumour cells *in vivo* and *in vitro* (Cavanagh 1996). The test material can be milk as well as blood serum (Gandy et al. 2001). Using blood, antibodies produced against the glycoprotein immunosuppressive factor of early pregnancy are isolated (Threlfall 1994). A serum pregnancy-associated glyco-

Table 5. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using progesterone.

Author	Days after AI	Material tested	Sensitivity (%)	Specificity (%)	Accuracy (%)
Dalmaso de Melo et al. 2020[1]	25	Blood	100.0	80.0	91.0
Dalmaso de Melo et al. 2020[2]	25	Blood	100	85.0	94.0
Holtz & Niggemeyer. 2019	21-49	Milk	96.4-98.8	40.1-68.2	84.8-99.1
Otava et al. 2007	19	Blood	100	53	82
Faustini et al. 2007	42-44	Milk	98.2	70.9	-
Dana et al. 2020	-	Blood	100	62.5	79.7
Ghaidan et al. 2019	-	Blood	92.3	80.1	84.8

[1] – heifers
[2] – cows

Table 6. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using EPF.

Author	Days after AI	Material tested	Sensitivity (%)	Specificity (%)	Accuracy (%)
Bastan et al. 2007	7-45	Blood (serum)	73.2	45.0	61.5
Ambrose et al. 2007	14	Blood (serum)	92.0	7.0	45.0
Ambrose et al. 2007	34-46	Blood (serum)	95.0	7.0	48.0
Ambrose et al. 2007	14	Milk	67.0	37.0	51.0
Ambrose et al. 2007	34-46	Milk	76.0	29.0	61.0

protein test (ECF™) is commercially available to determine pregnancy as early as 2 days after conception, but its effectiveness is questionable (Cordoba et al. 2001, Whisnant et al. 2001). In a study, using ultrasound (30 days after insemination) and *per rectum* examination (51 days after insemination) to diagnose pregnancy, it was found in 50% of inseminated cows. In contrast, when determining EPF from the same cows on day 30 after insemination in serum or milk, pregnancy was found in 100% and 37.5% of inseminated cows, respectively. In addition, test results for EPF using serum and milk, in 36.6% of cases, did not agree with each other. There was also a high – 47.5% frequency – of false positives for serum and 31.3% for milk (Gandy et al. 2001). In addition to pregnancy, elevated EPF levels can occur in tumours on the ovaries (Kumar Bharti and Jacob 2019). This method has not been widely used in practice, due to its high cost and the lack of possibility to carry out tests on a farm. Results for the use of this method in pregnancy diagnosis are shown in Table 6.

Interferon-tau concentration (IFN-τ) and expression of interferon-stimulated genes (ISGs)

Interferon-tau is produced by the embryo in cattle between 12-16 days after insemination (Mann et al. 1999). In ruminants, this polypeptide blocks the transcription of oestrogen receptor alpha and oxytocin receptors in endometrial cells (Spencer and Bazer 1996), regulating the expression of the enzymes cyclooxygenase-2 and prostaglandin F synthase (Xiao et al. 1999), which prevents the release of luteolytic prostaglandin. Part of IFN-τ passes from the uterus into the bloodstream (Oliveira et al. 2008), allowing it to be measured in blood serum. Its concentration in the blood is also influenced by the process of embryonic elongation (Kowalczyk et al. 2021). Due to the low concentrations of IFN-τ in the circulation, the expression level of interferon-tau stimulated genes (ISGs) in leukocytes can also be studied (Green et al. 2010). Despite many years of research, it has still not been possible to clearly establish the limiting level of IFN-τ required to maintain a pregnancy (Forde and Lonergan 2017), making unambiguous interpretation of the results difficult. Due to, among other things, the cost of the test, the test has not been widely used in practice. Results for the use of this method in pregnancy diagnosis are shown in Table 7.

Other methods

Thermography

The use of thermography in the diagnosis and treatment of animals has become increasingly popular in recent years (Eddy et al. 2001, McCafferty 2007). With the passage of time and the greater availability of thermographic equipment, attempts have been made to use this technique in the diagnosis of pregnancy in horses (Bowers et al. 2009, Domino et al. 2022),

Table 7. Comparison of sensitivity, specificity and accuracy of pregnancy diagnosis in cows using IFN-τ and ISGs.

Author	Days after AI	Material tested	Sensitivity (%)	Specificity (%)	Accuracy (%)
Dalmaso de Melo et al. 2020[1]	20	Blood	78.0	82.0	80.0
Dalmaso de Melo et al. 2020[2]	20	Blood	94.0	65.0	81.0
Dalmaso de Melo et al. 2020[3]	20	Blood	74.0	70.0	72.0
Dalmaso de Melo et al. 2020[4]	20	Blood	75.0	75.0	75.0
Panjaitan et al. 2021	18	Blood (serum)	60	71.4	66.7

[1] – heifers, marking ISG15 (Interferon-stimulated gene 15)
[2] – heifers, marking OAS1 (2'-5'-oligoadenylate synthetase 1)
[3] – heifers, marking ISG15
[4] – heifers, marking OAS1

sheep (Yazlik et al. 2020) and cattle (Radigonda et al. 2017, Olğaç et al. 2023), among others, these authors, using infrared thermography (ITA), observed no significant differences ($p>0.05$) in vulvar skin temperature in pregnant and non-pregnant cows. Other researchers (Olğaç et al. 2023) have proposed a method based on the evaluation of the difference in skin temperature measured with a thermal imaging device for two polygons located in the anal region (polygon 1) and the labia region (polygon 2). A ΔT°C value (T°C of polygon 2 - T°C of polygon 1) of ≤ 2.9°C was found to have a high correlation with pregnancy in cows as early as day 6 after artificial insemination ($p<0.001$). While a high correlation of temperature differences of the two polygons with pregnancy could be obtained in cows, no temperature difference could be determined in heifers for early pregnancies up to day 20 after insemination. The above studies were conducted on a relatively small number of cows. For this reason, with the current level of knowledge, they can hardly be considered binding. Further studies are needed to confirm the results obtained.

Fetal electrocardiography

The use of an electrocardiography (ECG) machine can also be helpful for the purposes of pregnancy diagnosis in cows. Its use for pregnancy confirmation began in the 1960s (Too et al. 1965). Several electrodes are used to obtain the best ECG recording. For cow ECG detection, they are placed on the left side of the body above the scapula and in the distal part of the sternum. To obtain an adequate ECG recording of the foetus, three electrodes are located on the left side of the cow's abdomen, near the intersection of a line drawn vertically from the fourth lumbar vertebra and a horizontal line drawn from the knee joint. The fetal ECG is then separated from the cow's ECG using appropriate software (Chen et al. 2000). Initially, the electrocardiograph was equipped with numerous electrodes and, according to the researchers, allowed confirmation of pregnancy with 100% accuracy from day 146 of pregnancy (Too et al. 1965). The same team successfully used this method to diagnose twin pregnancies from the 5th month of pregnancy (Kanagawa et al. 1965), as well as to determine the vital functions of the fetus after mid-pregnancy (Kanagawa et al. 1966, Steffen et al. 1995). A major advance and simplification was the development of a handy device that analysed the electrocardiographic recordings in addition to a phonogram. It looks like a telescope and processes the data collected by electrodes from the surface of the animal's body and the built-in phonogram in real time. According to the authors, diagnosis of pregnancy was possible from as early as six weeks after conception. Using the instrument, testing more than 2,000 in-calf and out-of-calf cows, it was possible to achieve a sensitivity of 89.4% and a specificity of 91.5%. However, according to the authors themselves, a sensitivity and specificity of 95% and the ability to test at least 60 cows per hour are required for commercial application of this method (Gargiulo et al. 2012). The fetal ECG signal depends on the position of the electrodes and the gestation period, which can cause problems in obtaining an adequate recording with different cow sizes and changing operators of the device. Furthermore, despite appropriate data processing, maternal QRS bands can be a source of misinterpretation of the fetal heart rate, especially for the untrained user (Chen et al. 2000). Despite many years of research, the devices described are not commercially available, nor do they provide basic data such as the sex of the foetus, its approximate age or information on the structures currently present on the ovaries.

Summary

Despite the numerous non-invasive methods for the early diagnosis of pregnancy in cows, ultrasound examination and *per rectum* palpation are still the most widely used. The advantages of these methods are the short examination time, the possibility of visualising the embryo/fetus, the low cost of the examination, as well as the precise assessment of the functional structures of the ovaries or the diagnosis of pathological changes within the uterus, enabling immediate veterinary intervention. This is supported by the speed of the examination – up to 100 cows per hour – and its relatively low cost (Jaśkowski et al. 2019). As shown above, non--invasive methods of pregnancy diagnosis are also associated with longer waiting times for test results, lack of added information (pathologies within the reproductive tract) and, with the exception of the PAG test, lower accuracy, which may affect the efficiency of reproduction. Some doubts may be raised regarding the welfare of the cows, as the collection of urine or blood requires contact between a third party and the animal, which, at least in theory, may cause distress to the animal. Given the arguments cited above, it appears that ultrasound and clinical *per rectum* examination will continue to be the predominant methods for diagnosing pregnancy in cows in the near future.

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