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Comparative study on certain early pregnancy diagnostic methods in Sahiwal cows

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Abstract
A study was conducted on 24 Sahiwal cows with an objective to compare and evaluate accuracy, sensitivity and specificity of certain early pregnancy diagnostic methods *i.e.*, Transrectal Ultrasonography (TRU), Serum Progesterone Assay (P4 assay) and Pregnancy Associated Glycoprotein (PAG) test on days 24, 28, 35 and 42 of post insemination. Confirmative pregnancy diagnosis was done by Transrectal Palpation on day 60 of post insemination. According to the results it was concluded that TRU is highly effective method for diagnosing early pregnancy as it is a direct method of pregnancy diagnosis in cows. However, serum P4 assay and PAG test could be used more accurately for diagnosing non-pregnant/ open cows at the earliest post insemination.

Keywords: Early pregnancy diagnosis, transrectal ultrasonography (TRU), serum progesterone assay (P4 assay) and pregnancy associated glycoprotein (PAG) test

Introduction
Early diagnosis of pregnancy has an important role in the reproductive management and the profitability in dairy farms (Khatti *et al*., 2021) [20]. In dairy cows, the optimum reproductive efficiency was required to achieve maximum lifetime production. Further, to make dairy farming economically viable and profitable, dairy cows must become pregnant with minimum Service period, carry the conceptus to term, deliver healthy calves, and produce milk on a regular basis (Rashmi *et al*., 2020) [29].

Pregnancy detection at an early stage in dairy cows forms a novel method for reducing service period (Pieterse *et al*., 1990; Rashmi *et al*., 2020 and Szenci, 2021) [27, 29, 31] and as an important reproductive management tool for optimising dairy cattle reproductive efficiency (DeVries, 2006) [10]. As a result, new technologies that detect non-pregnant dairy cows and heifers soon after artificial insemination play an important role in the management strategies to improve reproductive efficiency and profitability in commercial dairy farms (Fricke *et al*., 2016) [12]. Hence, the early pregnancy diagnosis becomes crucial to shorten the service period in cows, which need to be treated and/or rebred at the earliest.

The Sahiwal breed originated in the dry Punjab region which lies along the Indian-Pakistani border and it was considered as one of the best dairy breeds in India and Pakistan. It is tick-resistant, heat-tolerant and noted for its high resistance to parasites, both internal and external.

The direct method of pregnancy diagnosis involves the direct detection of the conceptus tissues and/or associated fluids, either manually by rectal palpation or visually via electronic instrumentation such as ultrasonography. At specific stages of pregnancy, secretions of the conceptus and corpus luteum such as early pregnancy factor (EPF), interferon-tau, estrone sulphate, pregnancy-associated glycoproteins (PAGs) and progesterone (P4) could be measured qualitatively or quantitatively (Balhara *et al*., 2013) [1]. A good early pregnancy test for dairy cattle should be accurate, sensitive, specific, cost effective, and easy to use in the field. (Fricke *et al*., 2016 and Pohler *et al*., 2016) [12, 28].

Transrectal, real time B-mode ultrasonography was suggested as an alternative to detect early pregnancy (Kastelic *et al.*, 1989; Kahn, 1990 and Szenci, 2021) [19, 18, 31] and found to be non-invasive, non-disruptive, reliable and results in time saving. It could be performed with minimal stress to the animal (Pierson and Ginther, 1984; Balhara *et al*., 2013 and Patel *et al*., 2016) [26, 1, 25]. Moreover, recording of fetus proper, fetal movements, heartbeat and other fetal parameters were also possible through ultrasonography (Curran *et al*., 1986 and Szenci, 2021) [7, 31].

It could also help in differentiation of normal and abnormal pregnancies (Chaffaux *et al.*, 1986 and Fissore *et al.*, 1986) [6, 11].

Progesterone (P4) concentration increases up to 14 days post estrus and continues to elevate up to day 21 after successful fertilization with specificity of 98.00 per cent (Nebel *et al*., 1987; Waldman, 1993; Fricke *et al*., 2016; Nepal *et al*., 2019 and Rashmi *et al*., 2020) [23, 32, 12, 24, 29].

Pregnancy-associated glycoproteins (PAGs) were considered to be pregnancy specific markers. The PAGs were found in the blood serum soon after implantation and pregnancy specific protein B (PSPB) was the first to be identified as PAG protein. PAGs were secreted from mono-nucleated and bi-nucleated trophoblastic cells of the outer epithelial layer in the placenta (Butler *et al*., 1982; Green *et al*., 2005 and Ghaidan *et al*., 2019) [5, 15, 14]. Wooding, 1992 [33] and Hameed *et al*., 2020 [16] reported that PAGs enter the maternal circulation as early as day 22 to 24 and reach levels required for accurate pregnancy diagnosis by day 28. A substantial increase in PAGs occur few weeks preceding parturition, peaks at calving and found in negligible level in maternal circulation at eight weeks postpartum (De Sousa *et al*., 2003; Green *et al*., 2005 and Szenci, 2021) [9, 15, 31]. The present study has been designed to conduct a comparative study on certain early pregnancy diagnostic methods in Sahiwal cows, with an objective to compare an accuracy, sensitivity, specificity of certain early pregnancy diagnostic methods and to evaluate more effective early pregnancy diagnostic method.

Materials and Methods

The study was carried out in Twenty-four Sahiwal cows aged between 2-6 years over a period of six months (from June 2022 to November 2022) at Dairy Experimental Station, Department of Livestock Farm Complex and Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad of P. V. Narsimha Rao Telangana Veterinary University and Pancharatan Dairy Farm, Malkaram village, Shamshabad Mandal, Rangareddy District, Telangana.

Three different early pregnancy diagnostic methods *i.e*., Transrectal Ultrasonography (TRU) with a portable, battery operated Ultrasound scanner with 5-7.5 MHz transrectal linear array transducer, Serum Progesterone Assay (P4 assay) with commercially available Bovine Progesterone GENLISATM ELISA kit, Pregnancy Associated Glycoprotein (PAG) test with Rapid Visual Pregnancy Test (RVPT) kit (IDEXX Laboratories, USA) were carried out in total twenty-four Sahiwal cows on days 24, 28, 35 and 42 of post insemination. Confirmative pregnancy diagnosis was done by Transrectal Palpation on day 60 of post insemination.

Results and Discussion

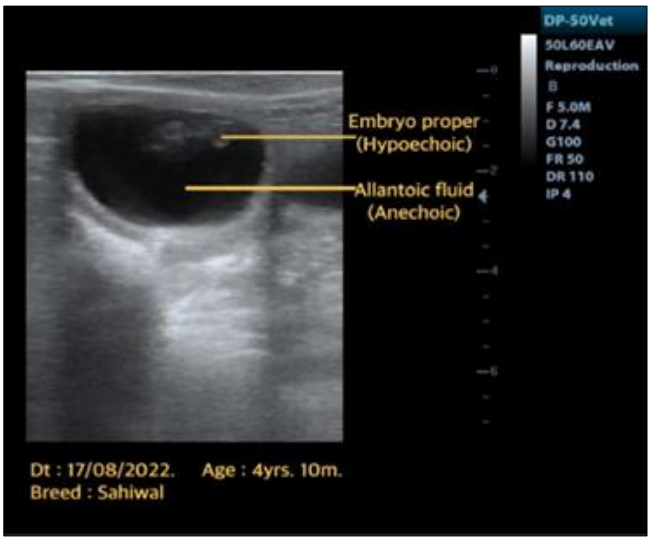

Fig 1: Picture showing the ultrasonographic image of gravid uterus of Sahiwal cow on day 24 of post insemination

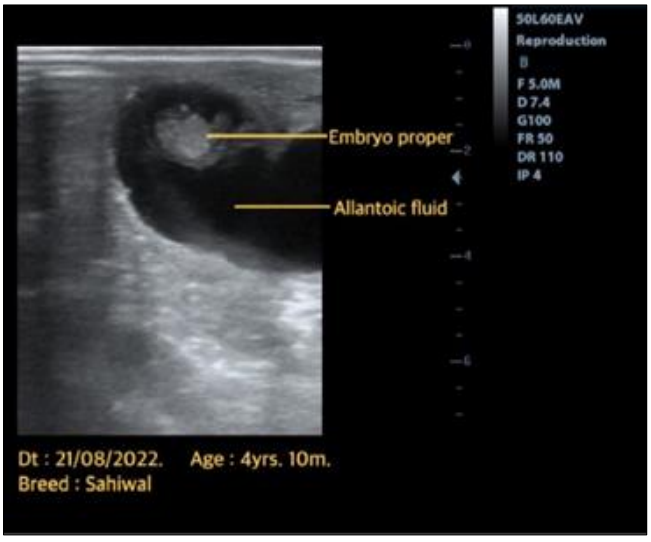

Fig 2: Picture showing the ultrasonographic image of gravid uterus of Sahiwal cow on day 28 of post insemination

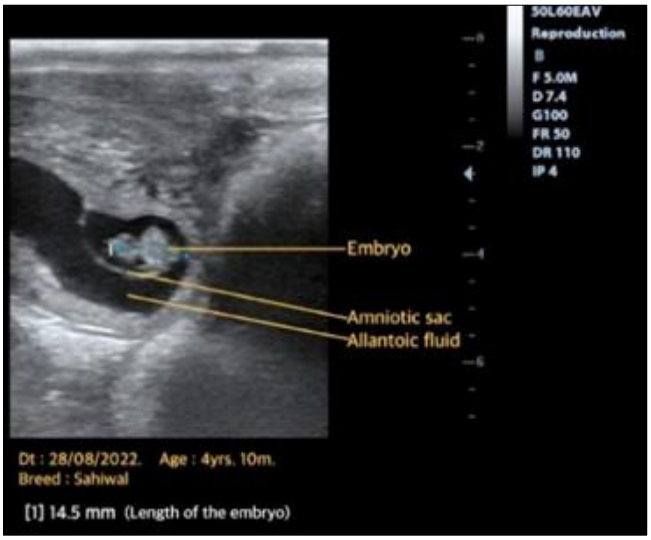

Fig 3: Picture showing the ultrasonographic image of gravid uterus of Sahiwal cow on day 35 of post insemination

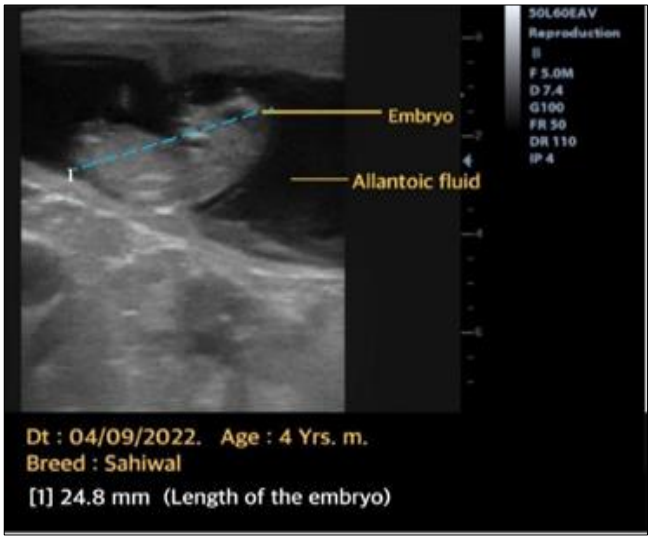

Fig 4: Picture showing the ultrasonographic image of gravid uterus of Sahiwal cow on day 42 of post insemination

Early pregnancy diagnosis was carried out in total 24 Sahiwal cows on days 24, 28, 35 and 42 of post insemination by Transrectal Ultrasonography (TRU). A portable, battery operated DRAMINISKI 4Vet Slim Ultrasound scanner with 5-7.5 MHz transrectal linear array transducer was used to scan the uterus for pregnancy diagnosis. Visualization of hypoechoic embryo proper within an anechoic allantoic fluid on day 24 (Fig. 1), hypoechoic C shaped embryo proper within an anechoic fluid with viable fetal heart beat on day 28 (Fig. 2), echogenic embryo measuring 13 to 17mm length with viable fetal heart beat on day 35 (Fig. 3)and embryo measuring 23 to 26mm length within an anechoic embryonic vesicle with viable fetal heart beat on 42 of post insemination (Fig. 4) were considered as positive for pregnancy by TRU (Curran *et al.*, 1986; Boyd *et al.*, 1990; Pieterse *et al.*, 1990 and Szenci, 2021) [7, 4, 27, 31].

Further, another early pregnancy diagnostic method the Serum Progesterone Assay (P4 assay) was employed in total 24 Sahiwal cows on days 24, 28, 35 and 42 of post insemination using ELISA technique. Further, the cows with the serum progesterone levels ≥ 2.00 ng/ml on day 24 of post insemination and ≥ 3.00 ng/ml on days 28, 35 and 42 of post insemination were considered as pregnant.

On the other hand, the IDEXX Rapid Visual Pregnancy Test (RVPT) Kit was used to detect the presence of Pregnancy Associated Glycoprotein (PAG) in total 24 Sahiwal cows blood serum on days 24, 28, 35 and 42 of post insemination. A positive pregnancy result was indicated by the development of blue colour in the sample wells, while a negative result was indicated by the absence of colour development.

Fig 5: Picture showing the results of PAG test using IDEXX Rapid Visual Pregnancy Test Kit. A) Pregnant- Development of Blue Colour, B) Non-Pregnant- No Colour Development

Finally, confirmatory pregnancy diagnosis was done by conducting Transrectal Palpation in total 24 Sahiwal cows on day 60 of post insemination and the signs like the asymmetry of uterine horns, double membrane slip and accumulation of allantoic fluid in the uterine lumen were considered for pregnancy confirmation. Diagnostic accuracy parameters

were calculated as per the procedures/ formulae described by Dana *et al*. (2021) [8]. Chi-square test was used to compare the sensitivity and specificity of the different pregnancy tests. Out of 24 Sahiwal cows subjected for transrectal palpation on day 60 of post insemination, 16 (66.67%) were diagnosed as pregnant while 8 (33.33%) as non-pregnant.

The relative efficacy of three different early pregnancy diagnostic methods in Sahiwal cows were studied and results are presented in Table 1. According to the present study, transrectal ultrasonography (TRU) had a higher sensitivity for diagnosing pregnancy (82.35%) on day 24 of post insemination when compared to serum P4 assay and PAG test (81.25%) which are in accordance with (Beal *et al*., 1992 and Holton *et al*., 2022) [2, 17]. However, P4 assay and PAG (87.50%) had more specificity for diagnosing non-pregnancy on day 24 of post insemination than TRU (85.71%).

Further, it was found that the sensitivity for diagnosing pregnancy on day 28 of post insemination through TRU was greater (93.75%) than serum P4 assay (82.35%) and PAG test (88.24%) as comparable with (Pieterse *et al*., 1990; Bonato *et al*., 1990 and Romano *et al*., 2006) [27, 3, 30], while the specificity for diagnosing non-pregnancy was recorded more for both serum P4 assay and PAG test (100.00%) than TRU (87.50%).

Table 1: Table showing the comparative accuracy of different early pregnancy diagnostic methods in Sahiwal cows on various days of gestation (n=24).

Method of early pregnancy diagnosis		Accuracy (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
TRU	Day 24	83.33	82.35	85.71	93.33	66.67
	Day 28	91.67	93.75	87.50	93.75	87.50
	Day 35	95.83	94.12	100.00	100.00	87.50
	Day 42	100.00	100.00	100.00	100.00	100.00
P4 assay	Day 24	83.33	81.25	87.50	92.86	70.00
	Day 28	87.50	82.35	100.00	100.00	70.00
	Day 35	100.00	100.00	100.00	100.00	100.00
	Day 42	100.00	100.00	100.00	100.00	100.00
PAG test	Day 24	83.33	81.25	87.50	92.86	70.00
	Day 28	91.67	88.24	100.00	100.00	77.78
	Day 35	100.00	100.00	100.00	100.00	100.00
	Day 42	100.00	100.00	100.00	100.00	100.00

Moreover, on day 35 of post insemination, TRU showed less sensitivity (94.12%) for diagnosing pregnancy when compared to both P4 assay and PAG test (100.00%) which is comparable with Nation *et al*. (2003) [22]. Further, all three pregnancy diagnosing methods *viz*., TRU, serum P4 assay and PAG tests exhibited overall 100.00 per cent specificity in diagnosing non-pregnancy on day 35. However, serum P4 assay and PAG tests on day 35 of post insemination were 100.00 per cent accurate in diagnosing both pregnancy and nonpregnancy is in agreement with Muhammd *et al*. (2000) [21].

On perusal of the results, it was concluded that on day 42 of post insemination, all the three methods of early pregnancy diagnosis *i.e*., TRU, P4 assay and PAG tests showed an overall diagnostic accuracy of 100.00 per cent for identifying both pregnancy and nonpregnancy corroborate with the reports by Friedrich and Holtz (2010) [13].

Conclusion

From the present study, it is concluded that TRU is highly effective method for diagnosing early pregnancy as it is a direct method of pregnancy diagnosis in Sahiwal cows. However, serum P4 assay and PAG test could be used more accurately for diagnosing non-pregnant/ open cows at the earliest post insemination.

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