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Dr. Pooja
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, International Institute of Veterinary Education and Research, Bahu Akbarpur, Rohtak, Haryana, India

Dr. Manish Kumar
M.V.Sc Scholar, Department of Veterinary Gynaecology and Obstetrics, Post Graduate Institute of Veterinary Education and Research, Jaipur, Rajasthan University of Veterinary and Animal Science, Bikaner, Rajasthan, India

Dr. Shikha Tamta
Assistant Professor, Department of Veterinary Public Health and Epidemiology, International Institute of Veterinary Education and Research, Bahu Akbarpur, Rohtak, Haryana, India

Corresponding Author:
Dr. Pooja
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, International Institute of Veterinary Education and Research, Bahu Akbarpur, Rohtak, Haryana, India

Laboratory methods of pregnancy diagnosis in buffalo and breeding efficiency

Dr. Pooja, Dr. Manish Kumar and Dr. Shikha Tamta

Abstract
Pregnancy diagnosis plays crucial part in the reproduction or breeding management of large animals as well as small animals and plays an important role in the reproduction management of ruminants since embryonic mortality has a substantial impact on the fertility of a herd. Embryonic death of fetus plays role or negative impact on fertility of animals. A large number of laboratory methods have been developed for pregnancy diagnosis in buffaloes including the detection of circulating hormones, pregnancy-associated molecules or chemical determination of these or other molecules in natural secretions (cervical mucus, urine or milk) from pregnant buffaloes, however, either their detection requires a specialized laboratory setup or their sensitivity and specificity is low limiting their widespread use. The process of implantation and the first few days following fertilisation are when most embryonic losses take place. So, it is very important to discriminate, with an early pregnancy diagnosis, non-pregnant from pregnant animals. Hormone analysis is simple technique for detection of pregnancy and used as an alternate as compare to rectal palpation or ultrasound. Large polymorphic family of placenta-expressed proteins has been reported for pregnancy diagnosis in ruminant species. Members of this family are named pregnancy-associated glycoproteins (PAG), being synthesized in the mono-and binucleate cells of the ruminant's trophectoderm. They will release in maternal blood circulation and easily diagnosed by laboratory methods. Due to large variety of expressed molecules and to large variations in the post-translational processing of the PAG, different immuno-systems present different ability to quantify the PAG released in blood.

Keywords: Pregnancy marker, hormonal methods, diagnosis, embryonic mortality

Introduction
India ranked first in total livestock production as per livestock production statistics of india-2022. The world buffalo population ranked first and increasing continuously and was estimated to be more than 194 million in 2010 as reported by FAO [FAOSTAT]. The process of implantation and the first few days following fertilisation are when most embryonic losses take place. Therefore, it is crucial to distinguish between non-pregnant and pregnant animals using an early pregnancy diagnostic. As an easier method than rectal palpation or ultrasound, hormone analysis may be used to identify pregnancy. Recently, a sizable polymorphism family of placenta-expressed proteins that may be utilised to diagnose pregnancy has been identified in ruminant species. Pregnancy-associated glycoproteins (PAG) are members of this family that are produced in the mono- and binucleate cells of the ruminant trophectoderm. Dairy is the single largest agricultural commodity contributing 5 percent of the Indian national economy and employing more than 8 crore farmers directly. More than 95 percent of the world population is found in Asia where buffalo population is highest in up and play a leading role in rural livestock production. When a man mates with a woman, he places sperm in her vagina, which is the precise definition of pregnancy. The embryo is created when the sperm and egg combine, becoming affixed to the womb's wall. The embryo develops inside a water bag and is connected to the womb's wall by a naval cord. If we talk regarding reproductive efficiency which is defined as the primary factor affecting productivity. Successful reproductive programmes depend on accurate diagnosis of pregnancy or non-pregnancy and the re-inclusion of non-pregnant buffalo into an appropriate breeding regimen. There have been created several techniques to enhance pregnancy detection. At the end trimester of pregnancy, the animal requires more feed and water for better reproduction. In order to diagnose pregnancy in cows,

non-return to oestrus and rectal probing of reproductive organs have been the most often used techniques. Due to the lower intensity of their oestrus behaviour compared to cows, buffaloes are much harder to observe during oestrus, so the absence of oestrus is misinterpreted when they don't become pregnant [Pawshe and Magee, 1994] [23].

Signs of pregnancy

kept close to home towards the end of the pregnancy and some form of shelter should be provided. They should be watched twice a day for signs that parturition is close. Cattle and buffalo in particular require a clean, well-ventilated area, ideally with a sand or grit surface on which appropriate bedding is laid. Do not confine a pregnant animal to a small space or tie it up all the time. Give her daily access to a field or garden.

Therefore, the probability of misdiagnosis of pregnant females by oestrus observation appears to be increased. This may be confounded by a small proportion of pregnant buffaloes expressing oestrus [Agarwal and Tomer, 1998] [1]. Only days 30 to 45 after mating are thought to be the most accurate times to diagnose pregnancy with transrectal palpation. The surgery may raise the chance of iatrogenic embryonic death, according to a few studies. However, the procedure doesn't offer any information regarding the embryo's or foetus' viability throughout the various stages of pregnancy Rectal palpation in buffaloes must be gentler than in cattle because the rectal mucosa is more delicate and more likely to bleed [Romano, 2001] [25].

Transrectal ultrasonography

Transrectal ultrasonography diagnosis in buffalo can be adopted successfully from Day 28-30 after service. At Day 30 it is possible to observed the fetal heart beats. The sensitivity reaching 100% from Day 31 after mating onward [Pawshe, 1994] [23]. The main advantage of the ultrasound scanning is that it can give an accurate diagnosis earlier than rectal palpation, exceeding palpation in the amount of information obtained from each animal. Anyhow, it is necessary restrain the animals and have a proficient operator.

Laboratory methods

Different laboratory tests have been developed and utilize reproductive hormones as indicators of presence of a viable pregnancy. Some are in detailed form below like Progesterone hormone, estrone sulphate etc.

Estrone sulphate

Estrone sulphate (E1S) is a synthetic steroid hormone that mimics the effects of oestrogen. Among other applications, it is utilised in menopausal hormone treatment. E1S itself is essentially biologically inactive, with less than 1% of the relative binding affinity of estradiol for the estrogen receptors (ERs), ERα and ERβ. The compound acts as a prodrug of estrone and more importantly of estradiol, the latter of which is a potent agonist of the ERs.[1] Hence, E1S is an estrogen. It is the primary oestrogen component in conjugated oestrogens (Premarin) and esterified oestrogens (Estratab, Menest) as the sodium salt (sodium estrone sulphate). The estrone sulfate is produced by the feto maternal axis. The hormone presents in urine, milk, blood and feces and act as an indicator. The probable timing at which the hormone present in serum is approximately is at day 150 of gestation [Eissa *et al*., 1995]. A positive test indicates a viable fetus. In buffalo species, the optimal day for estrone sulphate detection in the blood is day 150 of gestation. A viable foetus is shown by a positive test. After the fourth or fifth month of pregnancy, Hung and Prakash found that the amounts of estrone sulphate in buffalo plasma gradually increased. As a result, utilising the estrone sulphate test to diagnose pregnancy in buffalo is tardy. A negative result can indicate that a pregnancy is not occurring, but it does not rule out the onset of a pregnancy. Instead, this test enables the assurance of foetal viability in the latter two rounds of pregnancy.

Pregnancy-associated glycoproteins

PAG (also called pregnancy-specific protein B, pregnancy specific protein 60 and SBU-3 antigen) constitute a large family of glycoproteins expressed in the outer epithelial cell layer of the placenta of Eutherian species. Some of them are secreted in maternal blood starting at the point when the conceptus becomes more firmly attached to the uterine wall and placentomes start to form. They are produced by mono- and binucleate trophoblastic cells. The Pregnancy-associated glycoprotein family were isolated from cotyledons of ewe, cow, buffalo, goat, bison, elk and moose [Huang, 1999] [15]. PAG belong to the aspartic proteinase gene family. However, most PAG molecules are assumed to be enzymatically inactive due to key mutations within their binding cleft [28]. On the basis of molecular biological data, it is estimated that cattle, sheep and probably all ruminants possess many, possibly 100 or more, PAG genes [Green, 2000] [12]. 22 boPAG genes (boPAG-1 to boPAG-22) were cloned and completely sequenced in the bovine. Ovine (15 genes) [21, 29] and caprine animals (approximately 11 genes) have less PAG genes [Garbayo, 2000] [11].

Several bovines, ovine, caprine and buffalo closely related PAG molecules (63–87% N-terminal amino acid identities) have been made available and have been used to produce antisera for radioimmunoassay (RIA) development [Sousa, 1996] [27]. The measurement of circulating concentrations of PAG as a biochemical marker of pregnancy in various ruminant species is established. Recently different chromatography allowed identification of new PAG from buffalo placenta. At the same time Carvalho *et al*. identified PAG immunoreactivity in granules in BNC (binucleate cell) from buffalo.

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PAG concentrations in the blood are measured as a biochemical indicator of pregnancy in a variety of ruminant species [Carvalho *et al*., 2006] [7]. Heterologous PAG RIA devices were used to measure the PAG concentrations in several buffalo species.

In a study of Barbato *et al*., concentrations of pregnancy-associated glycoproteins (PAG) were determined in buffalo cows (*Bubalus bubalis*) by using three different RIA systems (RIA-497, RIA-706 andRIA-708). Blood samples were collected from Week 0until Week 28 of pregnancy, and from parturition until Week 10 postpartum. During pregnancy, concentrations of PAG were detectable at Week 6 by the use of the three above-mentioned RIA systems (3.9± 1.3 ng/mL, 9.7 ± 1.3 ng/mL and 9.9 ± 0.7 ng/mL, RIA-497, RIA-706 and RIA-708, respectively).Concentrations increased gradually until Week 28,reaching 39.6 ± 4.0 ng/mL (RIA-497), 50.5 ± 11.9ng/mL (RIA-706) and 68.2 ± 20.8 ng/mL (RIA-708).Over the whole gestation period, PAG concentrations determined by RIA-706 and RIA-708 were strongly correlated, RIA-708 giving the higher concentrations. At parturition, mean concentrations ranged from 34.9 ±4.0 (RIA-497) to 84.7 ± 10.6 ng/mL (RIA-708). Thereafter concentrations decreased rapidly, reaching very low levels (< 1.0 ng/mL) at Week 8 postpartum (Figure 5). So, PAG concentrations measured by three RIA systems showed distinct profiles from those previously described in bovine species, with higher concentrations measured by RIA-706 and RIA-708 at Week 6 after artificial insemination, and lower peripartum levels. These results suggest that the buffalo pregnancy proteins are better recognized by the antisera raised against the caprine PAG. Interestingly, by using these systems, concentration of PAG were remarkably distinct from those measured in cattle, reaching higher levels at the 6th week of pregnancy and low levels postpartum.

Progesterone hormone

Progesterone (P4) is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens and is the major progestogen in the body. Progesterone has a variety of important functions in the body. It is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid. Concentration of progesterone in blood at 20- and 24-days post breeding has been used as a tool for early pregnancy diagnosis [Arora and Pandey, 1982] [3]. But using high blood progesterone levels at 21 days to predict pregnancy was only 66.7% to 75% accurate. In general, a single progesterone analysis does not offer enough data to appropriately assess the pregnancy status. Progesterone from milk has been used to diagnose pregnancies accurately in buffalo. The factors which may contribute to misclassification are irregular cycles, early embryonic death, which varies from 19 to25 days in buffalo, the uterine pathology resulting in persistence of corpus luteum, luteal cysts, in correct timing of insemination and embryonic death occurred after day 24.

Hormonal interactions

In the presence of oestrogens, progesterone has a variety of physiological actions that are enhanced. The PR is induced or upregulated by oestrogens via oestrogen receptors (ERs). This may be seen, for instance, in the way oestrogens enable progesterone to mediate lobuloalveolar growth in breast tissue. Increased levels of progesterone effectively decrease aldosterone's ability to retain sodium, which causes natriuresis and a decrease in the amount of extracellular fluid.

Reproductive system

Progesterone has key effects via non-genomic signalling on human sperm as they migrate through the female tract before fertilization occurs, though the receptor(s) as yet remain unidentified. Detailed characterisation of the events occurring in sperm in response to progesterone has elucidated certain events including intracellular calcium transients and maintained changes, slow calcium oscillations, now thought to possibly regulate motility. It is produced by the ovaries. Progesterone has also been shown to demonstrate effects on octopus spermatozoa.

Progesterone is sometimes called the "hormone of pregnancy", and it has many roles relating to the development of the fetus

- Progesterone converts the endometrium to its secretory stage to prepare the uterus for implantation. At the same time progesterone affects the vaginal epithelium and cervical mucus, making it thick and impenetrable to sperm. Progesterone is anti-mitogenic in endometrial epithelial cells, and as such, mitigates the tropic effects of estrogen. If pregnancy does not occur, progesterone levels will decrease, leading to menstruation. Normal menstrual bleeding is progesterone-withdrawal bleeding. If ovulation does not occur and the corpus luteum does not develop, levels of progesterone may be low, leading to anovulatory dysfunctional uterine bleeding.
- During implantation and gestation, progesterone appears to decrease the maternal immune response to allow for the acceptance of the pregnancy.
- Progesterone decreases contractility of the uterine smooth muscle. This effect contributes to prevention of preterm labor.
- A drop in progesterone levels is possibly one step that facilitates the onset of labor.
- In addition, progesterone inhibits lactation during pregnancy. The fall in progesterone levels following delivery is one of the triggers for milk production.

Management in pregnant animals

- You must remember that a pregnant animal will need more feed and will benefit from the addition of some grain to the feed towards the end of pregnancy.
- All pregnant animals should be kept close to home towards the end of the pregnancy and some form of shelter should be provided.
- They should be watched twice a day for signs that parturition is close.
- In particular cattle and buffalo need a clean, well-ventilated place, preferably with a sand or grit floor on which suitable bedding is placed.
- Do not keep a pregnant animal constantly tied up or with little room to exercise in. Allow her some freedom in a field or yard each day.

Breeding efficiency

In the economics of producing cattle, regular breeding is a crucial factor. The typical aim is one calf each year, which can only be achieved by improving the animals' reproductive productivity. Therefore, in order for successful reproduction to take place, both males and females must be able to mate, become pregnant, care for, and deliver viable offspring. Both genetic and non-genetic variables influence breeding and reproductive effectiveness. The non-genetic influences include things like climate, diet, and animal management. The ability to reproduce differs across species and breeds as well as between animals of the same breed. Animals cannot function to their genetic potential, no matter how well they are fed or managed.

Factors effecting breeding efficiency

- **Calving age:** The animals chosen must not be overly young or underdeveloped, and selection for the ideal body weight, or 55 percent of mature weight, is required. Therefore, for maximum lifetime profit, the age at first calving should be 22.5 to 23.5 months when the age at first service is 14 to 15 months.

- **Embryonic losses:** Early embryonic loss is crucial between days 15 and 16 when the embryo is adequately developed to withstand uterine PGF2 secretion. Due to lack of attachment to the uterine wall, further late embryonic losses may occur throughout the period of 25 to 40 days following insemination.
- **Calving interval:** Reproduction after calving is based on the cow's uterine health and involution. Due to the drop in milk production, the number of calves produced per cow, and other factors, extending the intercooling interval from 12 to 14 months or more significantly reduces the financial return over feeding expenditures. Therefore, if the calving interval in cows and buffaloes exceeds 365 days and 395 days, respectively, there will be a sizable financial loss.
- **Inter service interval or interbreeding interval**
- **Period of the standing heat:** This is dependent on how well all cows can detect estrus. Cow fertility, however, is influenced by a variety of variables, including as the quality of the semen, how the semen straws are handled, how AI breeding is done, and when the insemination occurs.
- **Detection of estrus**
- **Proper timing of insemination:** The viable life of an egg after ovulation is thought to be fewer than 12 hours, unless it is fertilised, as sperm take between 6 and 10 hours to reach the bottom part of the oviduct.
- **Dry period**: A dairy cow's dry season is an essential part of their life. if the newborn calves' health or viability are adversely impacted by the gestational period and it cannot be reduced. During this time, nutrients are needed for both the developing foetus and the cow's maintenance. Typically, a lactation period of 50 days is ideal.
- **Handling of semen straws while AI:** Shaking the straw as it thaws at 37°C for at least 30 seconds will maintain maximum fertilisation rates. Once defrosted, inseminate right away to avoid suffering from heat or cold shock.
- **Fertility and season:** Due to decreased hunger, a cow's fertility is decreased by hot weather and heavy humidity. Blood estradiol levels, progesterone levels, follicular activity, and ovulation are all decreased by heat stress.
- **Skin health:** The estrogen receptor, as well as the progesterone receptor, have been detected in the skin, including in keratinocytes and fibroblasts. At menopause and thereafter, decreased levels of female sex hormones result in atrophy, thinning, and increased wrinkling of the skin and a reduction in skin elasticity, firmness, and strength. These skin changes constitute an acceleration in skin aging and are the result of decreased collagen content, irregularities in the morphology of epidermal skin cells, decreased ground substance between skin fibers, and reduced capillaries and blood flow. The skin also becomes more dry during menopause, which is due to reduced skin hydration and surface lipids (sebum production). Along with chronological aging and photoaging, estrogen deficiency in menopause is one of the three main factors that predominantly influences skin aging.
- Hormone replacement therapy, consisting of systemic treatment with estrogen alone or in combination with a progestogen, has well-documented and considerable beneficial effects on the skin of postmenopausal women. These benefits include increased skin collagen content, skin thickness and elasticity, and skin hydration and surface lipids. Topical estrogen has been found to have similar beneficial effects on the skin. In addition, a study has found that topical 2% progesterone cream significantly increases skin elasticity and firmness and observably decreases wrinkles in peri- and postmenopausal women. Skin hydration and surface lipids, on the other hand, did not significantly change with topical progesterone. These findings suggest that progesterone, like estrogen, also has beneficial effects on the skin, and may be independently protective against skin aging.

Conclusions
A crucial element of effective reproduction programmes. PAG concentrations in the peripheral maternal circulation have been measured in practise and utilised for both pregnancy confirmation and trophoblastic function monitoring. Veterinarians and breeders can benefit from the first component in managing reproduction, while researchers looking into variables influencing embryo and foetal mortality and embryo biotechnology can greatly benefit from the second. In the economics of producing cattle, regular breeding is a crucial factor. The typical aim is one calf each year, which can only be achieved by improving the animals' reproductive productivity. Therefore, in order for successful reproduction to take place, both males and females must be able to mate, become pregnant, care for, and deliver viable offspring. Both genetic and non-genetic variables influence breeding and reproductive effectiveness. Any dairy business' ability to produce enough milk, which is a direct result of animal reproduction, is what determines its ability to succeed. The interaction of genetic and non-genetic elements, which frequently declines as a result of subpar management, determines breeding efficiency. Selection, infectious disease management, dietary management, reproductive management, such as oestrous synchronisation, ovulation, and boosting male fertility, are only a few of the crucial areas for increasing animal fertility.

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