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Repeat breeding: Incidence, risk factors and diagnosis in buffaloes

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ABSTRACT

Repeat breeding in buffaloes was evaluated in terms of incidence, risk factors and diagnosis. The incidence of repeat breeding is low in buffaloes however in different studies the incidence varied from 0.70% to 30%. Because of seasonal suppression of fertility repeat breeding in buffaloes should be limited to the breeding season. Spring and winter calving, first parity, peri-parturient disease and lactation are significant risk factors for repeat breeding in buffaloes. The etiologies of repeat breeding in buffaloes can be failure of fertilization and early embryonic deaths. Only a few of causes of failure of fertilization have been identified in buffaloes. Ovulatory disturbances and ovarian cysts are uncommon in buffaloes and cysts have poor clinical manifestation. Endometritis is the common female cause of fertilization failures in buffaloes whereas poor semen quality and improper insemination are the bull side factors for fertilization failures. Early embryonic deaths are common in buffaloes mated/inseminated during the end of the breeding season due to a low luteal progesterone however embryonic deaths occur late (<25 days) in buffaloes. Diagnostic approaches for repeat breeding include vaginoscopic and transrectal examination and uterine cytology for genital health. More precise evaluations of the ovarian and uterine function can be obtained by ultrasonographic and hysteroscopic examinations performed sequentially however, precise diagnosis of the cause of repeat breeding seems difficult.

1. Introduction

Buffalo plays an important role in maintaining a sustainable food production system in the developing countries [1]. The productivity of buffaloes, however, remains low largely due to poor management of health, nutrition and breeding [2]. The major problems faced by buffalo breeders include poor reproductive efficiency and prolonged inter-calving intervals [3,4]. High incidences of fertility problems were associated with buffalo breeding [5]. Clinical evaluations have shown that anestrus and repeat breeding are the two major causes of infertility in buffaloes [6–9] however compared to cattle the incidence of repeat breeding is low in the buffalo (8.68% *vs.* 18.79%) [9]. A repeat breeder is generally defined as any cow that has not conceived after three or more services associated with true estrus [10]. Fertility problems in buffaloes are often not easily recognized; particularly studies on the repeat breeding syndrome are very few [11]. Buffaloes not conceiving after 3 or more services have been considered as repeat breeding [11,12] however, owing to seasonal suppression of fertility during hot summer months such considerations should be limited to the breeding season [10]. Repeat breeding (RB) syndrome is responsible for long service period and inter-calving interval thereby causing low milk and calf production resulting in to greater economic losses to dairy industry [7]. To curtail these losses exact and early diagnosis of the underlying etiology followed by timely interventions is a prerequisite [7]. The etiology of repeat breeding appears to be multifactorial and include uterine infections and reproductive tract abnormalities, hormonal dysfunction and nutritional inadequacies, and poor breeding and health management [10]. Clinical evaluations often depict the preponderance of genital infections (endometritis) in repeat breeding buffaloes [8,9,12,13]. During recent years many publications have appeared on the

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risk factors, etiology, and diagnostic approaches for repeat breeding in buffaloes and in this review the authors have addressed these issues.

2. Incidence

The incidence of repeat breeding appears to be low in buffaloes compared to cattle [9,10]. Incidence evaluations from clinical data (Table 1) have probably recorded higher incidence on account of the type of cases commonly presented (including buffaloes with low fertility during summer) to clinicians and not based on incidence in buffalo herds.

Variations in incidence can also be attributed to the heterogeneity of causes of the repeat breeder syndrome as well as the effect of locality and season [18].

3. Economic implications of repeat breeding

The economic success of dairy cattle and buffalo economy lies in proper and optimal reproductive rhythm of each individual cow and buffalo in the herd within normal physiological range [21]. Any deviation or prolongation in the breeding rhythm results in a progressive economic losses due to widening of the dry period and reduced calving and lactation during the life span of the animal [22]. Barren or infertile buffaloes mean a direct loss in milk production whereas reduced calf crop hamper the selection efficiency in long term dairy herd improvement [23]. Poor reproductive performance of the animals leads to economic losses due to reduced production and additional cost on management [24]. Sub-fertility, infertility and sterility is the outcome of impaired normal function, all of which result in economic losses due to anestrus, extended dry period, late maturity, decreased calving percentage and life time productivity of animal, increased cost of management and intense culling of the animals [25]. About 30.4% of cattle and buffaloes were culled mainly due to infertility, which incriminates direct losses to the farmers as well as to the genetic resource [26]. Reproductive disorders such as repeat breeding can lead to economic losses in terms of reduced fertility, low life time production, longer calving interval and increased culling in dairy cows [27] and buffaloes [28]. Calving intervals in buffaloes are longer compared to those in cows and buffaloes have a one month longer gestation period [4]. The calving intervals are prolonged on account of delayed resumption of postpartum estrus [29] and a further delay in conception due to repeat breeding would substantially increase these losses. Repeat breeding would contribute to significant economic losses with buffalo heifers which inherently evidence delayed puberty and higher age at first calving [30].

Table 1

Incidence of repeat breeding in different studies in buffaloes (%).

Breed	Incidence	References
Mehsani	6.00–30.00	[5]
Bihar	8.82	[14]
Murrah	5.00	[15]
Pakistani buffaloes	0.70	[16]
Egyptian buffaloes	4.34	[17]
Gujarat	6.35	[18]
Tamil Nadu	4.03	[19]
Murrah	11.04	[20]
Nepal	6.00	[12]
Uttar Pradesh	5.40	[15]

4. Risk factors

A large number of risk factors for repeat breeding have been described for cows including parity, peri-parturient disease, season, herd size, milk yield and poor fertility [31]. Similar descriptions for buffaloes are few and are mentioned.

4.1. Season

The buffalo is a considered a seasonally breeding species and females show a decline in reproductive activity during hot summer months in response to increasing day length [4]. Buffaloes in India and Pakistan evidence optimum fertility during cooler months of the year [32]. The seasonal decline in reproductive activity is manifested by a reduced incidence of estrous behavior, a decrease in the proportion of females that undergo regular estrous cycles and generally lower conception rate [15].

Higher calving frequencies during rainy and winter seasons [33] reflect maximum fertility of buffaloes during the months of September to December in India [34]. A few recent studies depicted negative effects of temperature humidity index (THI) on fertility traits in buffaloes [35,36]. High levels of circulating prolactin [37] are considered to mediate the effects of hot summer by suppressing LH and progesterone secretion. Relationships between corpus luteum vascularization, corpus luteum function, and pregnancy outcome of AI in buffaloes were consistent across the breeding season and transition period in previous study [38]. The pregnancy rates and embryonic mortality rates in buffaloes bred during the breeding period were 58.0% and 7.3% whereas they were 45.6% and 23.0% during the transition period [38] reflecting the effects of transition period on increase in embryonic mortalities and decrease in pregnancy rates.

The distinction between the breeding season and the transition period is the relatively low proportion of buffaloes that have optimum corpus luteum function and P4 concentrations required to establish a pregnancy during the transition period, which is manifested in a greater incidence of embryonic mortality and leads to repeat breeding [38].

4.2. Calving season

Season of calving influences the reproductive performance of buffaloes. Based on analysis of many reports it has been mentioned that buffaloes calving in the rainy and monsoon seasons had shorter anestrus period and higher fertility than other season calvers [29].

Reproductive disorders in buffaloes were common during summer and rainy seasons [39]. Significantly, higher incidence of repeat breeding was observed in buffaloes during the autumn season [40]. A higher incidence of poor fertility during autumn and summer probably occurs due to seasonal suppression of ovarian activity and increasing embryonic deaths during periods of increasing daylight length [41,42]. The first service to conception, number of services per conception and calving intervals were significantly lower in Nili Ravi buffaloes calving in summer (May to July) and autumn (August to

October) compared to those calving in spring (February to April) or winter (November to January) and Murrah [78,79] buffaloes calving during spring and winter [43].

4.3. Peri-parturient disorders and metabolic disorders

Postpartum metritis is one of the most important disorders in buffaloes [44], causing high economic losses due to prolonged days open and prolonged inter-calving intervals, resulting in culling. The incidence of uterine infections has been depicted to be higher in buffalo than cattle in a few studies [45]. Abnormal calvings and postpartum complications are a significant risk for development of fertility problems including repeat breeding in buffaloes [40]. Buffaloes with abnormal calvings and uterine health problems showed a higher odds ratio in favor of developing fertility problems [40]. Parturient and post parturient complications have negative impacts on fertility [46] and similar effects are seen with postpartum metabolic disorders in cows [47] however metabolic disorders are less frequent in the buffalo [48,49]. Buffaloes with retained placenta, dystocia or other parturient problem had significantly lower subsequent fertility [13,50], increased days open and increased number of services per conception.

4.4. Lactation

Repeat breeder buffaloes in the lactation herd revealed that lactating animals with high milk production required higher number of services per conception than non-lactating and low producing buffaloes [51]. The correlation coefficients were found significant between the number of services per conception and body weight at first service [51]. A higher proportion of buffaloes producing 3–6 kg of milk revealed repeat breeding [12]. However, in one study the association of conception rate with order of lactation, stage of lactation and milk yield was non-significant for 1 943 buffaloes studied [52]. Buffaloes producing more than 3 000 kg milk in lactation had the highest calving to conception period and those producing 1 000 kg in lactation had the lowest [34]. Milk production has been shown to have significant effects on conception rates in buffaloes inseminated artificially [52].

4.5. Parity order

The age of buffaloes in terms of parity order did not show any significant effect on repeat breeding [15]. Non-significant effect of parity on repeat breeding had also been reported [52], whereas, significant effect of parity order on the same trait was also noticed. In one study 60% of repeat breeding animals were buffalo heifers [12]. However, such results were not consistent across other studies (Table 2).

Higher number of services per conception in first parity buffalo heifers probably originate because of low intensity of estrus in buffalo heifers compared to adult buffaloes [53].

4.6. Nutrition

It has been largely demonstrated that nutrition affects reproductive performances in buffalo species [54]. However, these effects are more evident in countries where buffaloes calve during the most favorable periods of the year [55].

Table 2

Incidence of repeat breeding according to parity order in different studies in buffaloes (%).

Parity	Incidence	References
Heifer	3.85	[15]
	22.22	[14]
	60.00	[12]
1st	16.66, 59.00	[12,14]
2nd	7.15	[15]
	27.77	[14]
3rd	22.22	[14]
4th	11.11	[14]

Negative phenomena may be observed also in countries where the calving calendar is modified by applying the out of breeding season mating technique [56]. The most frequent nutritional errors are deficiency or surplus of energy, protein and carbohydrates and alteration of mineral equilibrium [54]. Dietary proteins affect blood and milk urea concentration in buffaloes [54]. Higher levels of urea in feed might affect the reproductive performance of buffaloes, due to higher levels of nitrogen concentration in the uterus [57]. Rumen protein degradability does not influence fertility in buffaloes due to a lower uterine diffusion of ammonia compared with cattle, because this species uses nitrogen better than cows [57]. Smooth ovaries condition was mainly due to the qualitative and quantitative deficiencies of the nutrition [58]. Similarly, silent estrus was attributed to under nourishment [58]. Deficiencies of calcium and phosphorous in repeat breeding buffaloes were marginal [59]. Similarly trace mineral concentrations of zinc [17] and copper [17,60] were found to be lower in repeat breeding buffaloes.

5. Etiology

The etiology of repeat breeding has been multifactorial. The causes of repeat breeding have been classified in cows in a number of ways, yet failure of fertilization and early embryonic deaths had been one of the oldest classifications of the etiologies [10]. The cause of repeat breeding may lie with the cow or the bull or a combination of these [10,31] and external factors such as environmental stress and poor breeding management [31]. The causes of repeat breeding in buffaloes are discussed under the headings of failure of fertilization and early embryonic deaths.

5.1. Failure of fertilization

Studies in cattle have shown that failure of fertilization accounts for a low proportion (10%–20%) of pregnancy losses during the first 21 days post insemination [61]. However, similar descriptions for buffaloes are not available. Moreover, studies evaluating the causes of fertilization failures are very few and suggest factors possible in the cow (ovulation failure, oviductal obstructions, abnormal ova and endometritis). The cause of fertilization failure can also lie with the bull and the technique and timing of insemination when using artificial insemination. Differential fertility of buffalo bulls and the season of semen collection appear important in the buffalo [62] with semen fertility being optimal during cooler months [63]. Ovulatory disturbances such as anovulation and delayed ovulation have been recorded in a few studies in buffaloes

[7,10] with resultant repeat breeding. The possible causes of fertilization failures in buffaloes are mentioned in lieu of their existence either with the female buffaloes, buffalo bulls and the breeding management although establishing clear etiology in repeat breeding cows is often difficult as concomitant and overlapping causes are often existent [31].

5.1.1. Female buffaloes

Ovulatory disturbances: Delayed ovulation, anovulation and ovarian cysts are less frequent in the buffalo [49] yet can result in fertilization failures. Clinical studies recorded the incidence of 0.5%–1.48% ovarian cysts in buffaloes [64] with poor clinical manifestation.

Oviductal obstructions and adhesions: Oviductal obstructions that probably prevent fertilization can originate from pathologies in the oviduct such as hydrosalpinx, pyosalpinx, salpingitis [65] and stenosis or growths in the oviduct [49] however most studies have utilized abattoir derived genitalia and clinical descriptions are few.

Ovarobursal adhesions have been mentioned in a few clinical descriptions with incidence varying from 0.04% [5] to 6.4% [13,65] yet abattoir studies reflect a higher incidence. Ovarobursal adhesions affect fertility as they interfere with tubal motility [49].

Endometritis: The negative effects of endometritis on fertility are mediated directly by bacterial endotoxins or indirectly by inflammatory mediators such as cytokines, nitric oxide and oxidative stress affecting sperm, ovarian, uterine and embryonic function [66]. Endometritis can affect sperm motility and function and results in increased sperm phagocytosis [66] or poor development of resultant zygotes. Endometritis is known to affect luteal development and nitric oxide concentrations in the buffalo [67] and thus can also result in embryonic mortalities besides fertilization failures. Oocytes exposed to bacterial toxins are less likely to develop to the blastocyst stage. Increased sperm phagocytosis is likely due to the increased influx of polymorphonuclear leucocytes in the lumen of endometritis affected buffaloes [68–71]. Moreover, increased oxidative stress has been recorded in buffaloes with endometritis [17,72]. In a recent study it was shown that the xanthine oxidase activity was significantly enhanced in Murrah buffaloes with endometritis [73]. Likewise significant differences in the toll-like receptors in the endometrium of endometritis affected buffaloes were recorded [74]. Histopathological studies have revealed denudation of endometrial epithelium, edema of connective tissue and scanty uterine glands in the endometrium of endometritis affected buffaloes [70]. An altered endometrial morphology might account for fertilization/pregnancy failures and an altered ovarian function. A high proportion (70.59%) of buffaloes with clinical endometritis suffered from ovarian inactivity [72]. The reported clinical incidence of endometritis appears to be very high (2.4%–20.68%) and clinical studies have recorded endometritis to be the most frequent disorder in buffaloes [13,76].

An important cause of fertilization failure appears to be the oocyte quality. The effects of postpartum disease on oocyte quality are poorly understood [75].

5.1.2. Bull factors

Repeat breeding can originate because of factors related to the bull and semen [31]. Differential fertility between buffalo bulls does exist [62,77] and appear to be an important determinant affecting conception rates to insemination or natural mating of buffaloes. The age of buffalo bull affects the semen volume and the proportion of abnormal spermatozoa; with adult buffalo bulls producing the highest volume and lowest abnormal sperms [78]. The bull breed, semen type (liquid or frozen), quality and source have marked effects on the conception rates [79]. An important consideration with buffalo bulls used as semen donors for AI appears to be the season of semen collection as semen collected during hot summer months has been shown to have lower fertility [63,78] and such semen can contribute to repeat breeding. Infectious diseases such as Campylobacter have been shown to be existent in buffalo bulls [80] and could be a probable reason for early embryonic deaths and repeat breeding in female buffaloes.

5.1.3. Breeding management

With the increasing use of AI in buffaloes important considerations for optimum fertility include insemination technique, time of insemination and site of semen deposition. The conception rate varies significantly between the inseminators [52]. Incorrect inseminations relative to estrus were performed in 30.67% of buffaloes in one study [81] and many repeat breeding buffaloes at insemination had plasma progesterone profiles greater than 1 ng/mL suggesting that buffaloes were inseminated at the wrong time [81]. A problem with buffalo AI is the poor estrus expression and lack of efficient means to detect estrus. Insemination relative to ovulation also appears important to achieve high fertility [82]. Buffaloes that ovulated after 96 h showed a lower fertility rate, and the presence of estrual mucus favored the conception rate in heifers only. Inseminations performed at the wrong time resulted in poor fertility in buffaloes [83]. Moreover, this depended upon the skills of the inseminator and the first AI conception rates varied from 25.40% to 37.83% for the different inseminators [83].

5.2. Early embryonic deaths

Embryonic mortality occurs in cattle mostly within the first 2–3 weeks of gestation [61,84] and accounts for 45% of pregnancy failures during this time. Low concentrations of circulating progesterone appear to be pivotal to embryonic deaths in cattle due to failure of endometrial and embryonic signal exchanges [85]. Other possible reasons could be genetic selection of cows for high production [61], environmental stress [85], shipping and heat stress [86] and uterine infections with pathogenic microbes [87]. For buffaloes low luteal progesterone and poor corpus luteum development [38,88] during periods of increasing daylight length contribute to 8.8%–21.8% of embryonic mortalities in buffaloes that were naturally mated [89]. A recent study mentioned that total luteal cells and progesterone content per corpus luteum during the mid luteal stage in the buffalo is less than cattle suggesting inherent luteal deficiency in buffalo [90].

The embryonic losses in buffaloes that were inseminated following estrus synchronization during sexual decline in reproductive function were 20%–40% [41,42,91]. Rearing systems that provide adequate cooling during the hot summer months resulted in significantly lower embryonic mortalities [92]. Other studies revealed that the proportion of embryonic mortalities was not affected by age, parity or lactation stage [41,42]. Infectious agents were mentioned only for 2%–8% of the embryonic losses in buffaloes [41,42]. Higher proportion of embryonic mortalities in buffaloes occur between day 28–60 [91,93,94] hence presence of an embryo on day 25–30 and its disappearance at day 45 or day 90 is considered embryonic mortality [41,42,91,93]. Luteal progesterone appears to play a fundamental role in embryonic development in the buffalo [95]. However, complex interactions between the embryo/fetus and mother modulate the proteins in the uterine lumen [96,97] and transcriptome profile expression [98] during early bubaline pregnancy necessary for continuance of pregnancy [99]. These studies have shown that at day 25 post mating buffalo embryos with retarded growth had differential expression of several proteins [97] and altered gene expression [98] compared to normal embryos. Another recent study [100] evidenced that the early development of corpus luteum at day 5 is related to the increased likelihood of pregnancy in buffaloes. It thus appears that normal bubaline embryonic growth requires an early development of luteal progesterone and its proper maintenance till the implantation period (day 25) [101] and then throughout gestation. Lower luteal progesterone at day 5 and day 25 appear to be crucial in initiating embryonic deaths. A seasonal increase in embryonic deaths during the transitional period (non-breeding season) has been recorded in buffaloes [38]. Microbes such as mycoplasma, listeria and fungi have been isolated from repeat breeding buffaloes hence their role in failure of fertilization and embryonic deaths is possible.

6. Diagnostic approaches

Diagnostic approaches for repeat breeding cows and buffaloes have been described in detail in a previous review [10]. Diagnosing the cause of pregnancy failures in individual animals is often extremely difficult and evaluations are oriented towards evaluating the health of the genital tract, physiological functioning of the ovaries and sequential growth and development of the embryo.

A combination of visual estimates, transrectal palpation and transrectal ultrasonography are suggested [10]. More specialized techniques such as endoscopy, metabolic profiles, uterine biopsies and tubal patency testing suggested previously [10] should be reserved for pathologies in the genital tract not easily traceable. Ovarian and oviductal pathologies such as tumors, pyosalpinx and oviductal obstructions are often not detectable with routine techniques and failure to regain fertility following medical or surgical therapy often limits their use [49] and the value of the affected animal.

6.1. Evaluating genital health

Visual estimations of cervico-vaginal mucus is often the first diagnostic method of finding infection in the genital tract of cows; however, due to lower quantity of mucus secretions during estrus in buffaloes [4] and tendency of buffaloes to evidence estrus during night hours [2] visual appraisal of cervico-vaginal mucus appears less efficient. Manual squeezing of genital tract (Uterus) is suggested for collection of cervico-vaginal mucus [10]. Alternatively, mucus can be retrieved from the vagina using sterile glass pipettes.

Cervico-vaginal mucus has been studied in terms of viscous, thin and thick consistency [10]. The mucus was found to be viscous in 38.33%, thin in 50.0% and thick in 11.67% of the buffaloes [102]. The percentage of conception was higher among buffaloes having thin consistency of cervical mucus than those having viscous or thick consistency [14,102]. Clear thin mucus showing typical fern pattern resulted in maximum repeat breeding buffaloes to conceive [103]. The pH of cervico-vaginal mucus of repeat breeding buffaloes was alkaline (8.02 ± 0.11) [14].

Vaginoscopic examination is helpful in evaluating the health of the vagina and cervix and also secretions from the uterus accumulating in the vagina [13]. Such an examination can also help in finding cervicitis or vaginitis [12] or changes in cervical morphology [5] which could be a possible cause of repeat breeding especially in natural matings. Anatomic abnormalities such as kinked cervix [5] that can affect fertility can be identified with transrectal palpations and vaginoscopic examinations.

A wide variety of microbes have been isolated from repeat breeding buffaloes including *Salmonella*, *Staphylococcus*, *Corynebacteria*, *Psuedomonas* and *Escherichia coli (E. coli)* [71,104–106] however their significance is important only when samples have been collected properly [10]. Often these organisms are present collectively with anaerobic bacteria like *Fusiformis necrophorus (F. necrophorus)* and *Bacteroids* species [107] and rarely fungi are also present.

Uterine biopsies can reveal the actual status of endometrium and its glands [74,108]; however, the invasive nature of the technique limits its clinical use routinely. A valid alternative to detect uterine infection in cows [109] and buffaloes [68,70] could be the cytological evaluation of uterine fluids collected by aspiration of uterine contents or uterine lavage using a cytobrush or Foley catheter. The proportion of polymorphonuclear cells was significantly higher in buffaloes with endometritis (45.62%) [68] and so were the neutrophil counts [70]. The practical utility of such a technique suffers from a lack of proportion of uterine cells diagnostic of endometritis in cows [109] due to wide variation in the proportion of cells on different days postpartum and thus the technique has not become popular among clinicians.

Ultrasonographic features diagnostic of endometritis include increased endometrial thickness and increased intraluminal uterine fluid accumulation [110] however in order to obtain high precision operators need sufficient experience. Ultrasonographic examination of the genital tract can also identify other uterine pathologies such as hydrometra and mucometra [111] that could be a possible reason for repeat breeding. Techniques such as hysteroscopy [112,113] can provide additional information about the uterine lumen such as hemorrhages not detectable by ultrasonography however the validity of such findings are yet to be known and the high cost of such techniques limit their current use.

6.2. Evaluating ovarian function

Two ovarian events appear to be important in repeat breeding buffaloes. First the timely ovulation in relation to insemination and second the formation and functioning of the corpus luteum. Transrectal palpation findings appear to suffer from lower accuracy on account of smaller ovarian dimensions in the buffalo [109] and thus transrectal ultrasonography appear to be a better diagnostic tool to monitor ovarian function including ovulation and corpus luteum formation in the buffalo. Ovulation can be monitored by frequent ultrasonographic examinations (6–12 h after estrus) and visualization of the disruption of ovulatory follicle [10]. The formation of corpus luteum can be more efficiently determined by ultrasonographic examination on day 5 post estrus [100]. Greater development of corpus luteum on day 5 is related to the increased likelihood of pregnancy in the buffalo [100]. Additional information on ovarian blood flow that can be helpful in predicting embryonic mortality can be gained by color Doppler ultrasonography [114]. Plasma progesterone profiles have been used in many studies to evaluate corpus luteum function and progesterone profiles greater than 1 ng/mL during mid luteal phase (Day 11–15) [115] suggest proper corpus luteum function [116]. A recent study [90] mentioned lower luteal function in the buffalo compared to cattle based on total luteal cells and progesterone content per corpus luteum during the mid luteal phase.

The determination of ovarian and oviductal pathologies that could be a possible reason for repeat breeding in buffalo (mentioned previously) can be accurate by transrectal ultrasonography when the pathological structures over ovaries are large in size and bilateral [49].

6.3. Evaluation of embryonic deaths

Embryonic deaths have been commonly evaluated by using sequential transrectal ultrasonography [41,42,93,94,117] and the disappearance of a previously visible fetus and/or fluids suggest embryonic death. Such estimations are difficult with transrectal palpations as most bubaline embryonic deaths occur between days 25–45. Low luteal progesterone is existent in buffaloes with embryonic deaths [91] however, using single or sequential progesterone profiles to predict embryonic deaths seem to be inappropriate.

6.4. Other evaluations

6.4.1. Serum biochemical profile

Variations in circulating levels of biochemical's have been recorded in repeat breeding buffaloes (Table 3) however, using serum biochemical profile or trace mineral status to predict potential fertility is far from perfect [10] and such evaluations can possibly be used to predict deficiencies in representative samples from large herds.

Table 3

Variations in circulating level of biochemical's recorded in repeat breeder buffaloes in different studies.

Serum biochemical profile	Repeat breeder	Cyclic buffaloes	References
Total protein	Lower	Higher	[51]
	Equal	Equal	[120]
Calcium	Lower	Higher	[59,118]
Glucose	Lower	Higher	[51]
	Equal	Equal	[118]
Creatinine	Higher	Lower	[51]
Inorganic phosphorous	Lower	Higher	[51,59,118]
Zinc	Lower	Higher	[17,51,118,119]
	Equal	Equal	[60]
Copper	Lower	Higher	[17,60,118,119]
Iron	Lower	Higher	[17,118,119]
Cholesterol	Equal	Equal	[120]

6.4.2. Level of oxidant and antioxidant markers

Oxidant/antioxidant markers in repeat breeding buffalo–cows serum have shown increased malondialdehyde (MDA) and nitric oxide (NO) and decreased catalase (CAT), superoxide dismutase (SOD), ascorbic acid (ASCA), reduced glutathione (GSH-R) and total antioxidant capacity (TAC) [17]. It is well known that in a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. When the balance is disrupted towards an over abundance of ROS, oxidative stress (OS) occurs. Also, ROS have a role in pathological processes involving the female reproductive tract, and it affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy [121]. Repeat breeding buffalo–cows showed increased MDA and NO and decreased CAT, SOD, ASCA, GSH-R and TAC [67,72]. The use of these markers in repeat breeding buffaloes is currently limited.

Conflict of interest statement

We declare that we have no conflict of interest.

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