Clinical Prespective Of Repeat Breeding Syndrome In Buffaloes

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ABSTRACT: Local meat production in Egypt is in continuous decrease and can not meet the local market requirement. So this study was designed to throw light on true repeat breeding syndrome (RBS) as one of the reproductive disorders that hinders the buffalo meat and milk production. A field survey was carried out on 1358 female buffaloes which were subjected to clinical and gynecological examination , and blood samples were collected for carrying out some relevant analyses. Treatment trials were practiced using different ways to control the condition and the economic impact of this syndrome has been studied. Results revealed that the incidence of clinical repeat breeding (RB) in the examined buffalo cows was 4.34 %. Typical repeat breeders represented 7.25 % of total reproductive disorders in female buffaloes. Serum progesterone level was 1.44 ± 0.39 and 3.66±0.84 in RB and normal buffaloes (NB), respectively. Oxidant/antioxidant markers in RB buffalo-cows showed increased malondialdehyde (MDA) and nitric oxide (NO) and decreased catalase (CAT), superoxide dismutase (SOD), ascorbic acid (ASCA), reduced glutathione (R-GSH) and total antioxidant capacity (TAC). Serum zinc, copper,iron and selenium values were lower in repeat breeder cows compared to normal animals. Repeat breeder buffalo-cows responded to the treatments with mineral mixture, GnRH and Lugol's solution with recovery rates; 63.64, 61.54 and 60.00%, respectively. The study concluded that special care should be paid for food additives to control this syndrome.

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1. INTRODUCTION

Currently, the incidence of infertility becomes relatively increased with consequent reduction of productivity of farm animals. According to global Agricultural Information Network report for 2010, the total number of Egyptian cattle and buffalo decreased from 6.256 million head in 2008 to 6.248 million in 2009 and it is expected to decline in 2010 to level lower than 2009 because of many problems that continue to limit the growth of the animal production industry [1]. The price of domestic beef increased dramatically and the imported frozen meat and liver are still important sources of protein in domestic market. The average per capita consumption of red meat including variety meats is estimated at 8.5 kilograms per year, which is quite low compared to consumption levels in other countries. The lower consumption is mainly due to limited local production combined with lower per capita income [1].

Although buffalo constitutes 49% of the above mentioned number, it is the source for high quality milk (65% of milk production), lean meat (33.9% of meat production) and preferred by most of Egyptians. However, in Egypt and most of developing countries having buffalo population, these animals are mostly raised in small holder farms under hard socioeconomic circumstances [2].

Reproductive disorders, poor nutrition, parasitic infection are the main constraints of buffalo development. Ovarian inactivity, silent heat, endometritis and repeat breeding are the main reproductive disorders in buffaloes in Egypt [3].

Typical repeat breeding (RB) is defined as the animal that did not conceive after three or more consecutive inseminations, despite; it comes normally in heat and shows clear estrous signs with no clinical detectable reproductive disorders [4].

The objective of this study was to throw light on typical repeat breeding syndrome in Egyptian buffaloes with emphasis on the oxidative status and application of some field treatment trials. Also, economic impact of this syndrome has been investigated.

2. MATERIALS AND METHODS

Animals

The current study was conducted on 1358 mature polyparous buffalo cows randomly selected from small-scale holders at Al Sherkia governorate, lower Egypt during 2008-2010. These animals were fed on Barseem (*Trifolium alexandrinum*), wheat or rice straw and a few amount of concentrate mixture. Based on owner complains, case history, general health condition and the gynecological examination, animals were categorized into two groups; the first group (G1) bred and conceived normally after no more than 3 inseminations. The second group (G2) was those animals which did not conceive after three or more inseminations, despite no clinically detectable reproductive disorders were observed (Typical repeat breeders). Gynecological examination was carried out through rectal palpation aided by ultrasonography machine (PiaMedical Falcs e`Saote, Netherlands) with an endorectal linear array of 8.6 M hertz to register the reproductive status and/or disorders.

Sampling and Analysis:

Blood samples were drawn from the jugular vein of each animal, in tubes with and without EDTA. Serum was separated after centrifugation and stored at −20 °C until analysis. Serum progesterone level was assayed by ELISA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg/ml with inter- and intra- run precision coefficient of variations of 2.9 and 4.85, respectively [5]. The concentrations of malondialdehyde (MDA) [6], nitric oxide(NO) [7], catalase (CAT))[8], ascorbic acid (ASC) [9], superoxide dismutase (SOD) [10] and total antioxidants(TAC) [11] in the serum and glutathione reduced (GSH-R) [12] in the whole blood were determined by colorimetric methods using chemical kits (Biomed Egypt) and Shimdzu UV 240 spectrophotometer. Zinc, copper, iron and selenium concentrations were determined using atomic absorption spectrophotometry (Perkin Elmer, 2380) as outlined by [13]

Treatment trials:

A total number of 34 repeat breeder buffalo cows was subjected to one of the following treatments: 1- No treatment at all and kept as the control group (n=5).

2- Lugol's Iodine solution (0.5 – 2%) as a vaginal wash for 3 successive weeks (n=5).

3- Receiving 20 g from a mixture of minerals, vitamins and Lasalocids® in their ration for 10 successive days. This mixture was prepared in the laboratory by throuogh mixing of 20 g of zinc sulphate , 6.25 g of copper sulphate, 1.5 g potassium iodide, 30 mg sodium selenite, 200 g AD3E and 5 g Lasalocids®, (F-Hoffman-La Roche,Basle, Swizerland) and sodium phosphate dibasic up to 1 kg [2] (n=11)

4- Receiving an intramuscular injection of GnRH (Receptal, Hoechst Roussel Vet GmbH) (n=13)

Treatments were carried out according to the instruction of manufacturing companies. Animals were followed up during the next weeks for conception.

Economic evaluation:

Economic losses were calculated on light of decreased calf crop, prolonged calving intervals, decreased milk production and veterinary intervention services, cost of the used drugs as well as cost of repeated AI.

Statistical analysis:

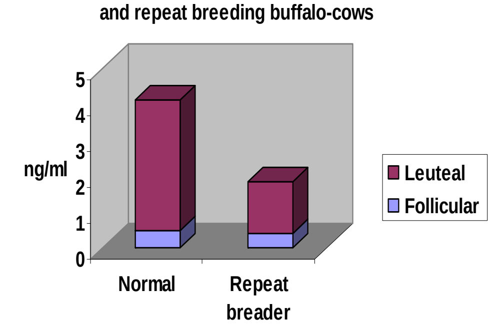
The data were computed and statistically analyzed using PSS-10.5 software package [14].

3- RESULTS

3.1. Incidence

The present study declared that 59 (4.34%) out of 1358 examined polyparous buffalo- cows are typical repeat breeders with an average body condition and good health status. Moreover, this syndrome represented 7.25% of the total recorded reproductive disorders (813).

Fig.1: Serum progesterone level in normal and repeat breeding buffalo-cows



3.2. Reproductive aspect:

The studied buffalo cows came normally in heat inseminated in the proper time with normal proven fertile bulls and came in heat again after 20 – 22 days during the breeding season of buffaloes (September– March). Ultrasonographic examination revealed that such animals showed no detectable clinical reproductive disorders with the corresponding normal physiological structure during the different stages of the estrous cycle. Serum progesterone level (Fig. 1) was significantly ($P< 0.05$) low (0.44 ± 0.39 ng/ml) during the mid -luteal phase of the estrous cycle in repeat breeder animals compared to normal animals **(**3.66±0.84 ng /ml).

3.3. Oxidative status:

Table 1 show the oxidant/ antioxidant status of the investigated animals. It was found that MDA and NO values were significantly high ($P< 0.01$) while, ASC, GSH-R, Zn, Cu, Fe and Se values were significantly($P< 0.01$) low in repeat breeder buffalo-cows compared to normal animals.

Table (1): Oxidant / antioxidants concentrations in repeat breeder buffalo- cows (Mean ± SE).

Oxidant/antioxidant	Normal cows (N=10)	Repeat breeder buffalo-cows (N=10)
MDA (mmol/ml)	1.98 ± 0.09	3.70 ± 0.48 **
NO (mmol/L)	15.55 ± 1.58	25.17 ±0.85**
CAT (U/ml)	2.28 ± 0.4	1.99 ±0.10
ASC (µg/dl)	132.17 ± 5.12	95.16 ±2.37**
SOD (U/ml)	338.16 ± 7.11	332.12±16.14
GSH-R (mmol/L)	6.38 ± 0.11	2.66 ±0.09**
TAC (mmol/L)	1.43 ± 0.08	0.46 ±0.50
Zinc (µg/dl)	139.11 ± 2.17	120.21 ± 5.20**
Copper (µg/dl)	78.65 ± 0.13	68.33 ± 2.05**
Iron (µg/dl)	168.40 ± 4.11	152.13±2.05**
Selenium (µg/L)	144.85 ± 0.34	130.12±2.01**

** $P< 0.01$

3.4. Treatment trials:

Field trails to treat the typical repeat breeding syndrome using Lugol's solution, mineral mixture and GnRH indicated that 60 – 63 % of the treated animals get conceived as indicated by gynecological examination in 40 – 60 days later, while no animal from the untreated group get conceived. It was found that mineral mixture- treated group gives the highest response .

Table (2): Treatment trials for repeat breeder buffalo-cows (Mean ± SE).

Treatment	Repeat breeder-cows	Recovered animals	Recovery (%)
No treatment	5	0	00
Lugol's solution	5	3	60
Mineral mixture	11	7	63.64
GnRH	13	8	61.54

3.5. Economic evaluation

In the present study, economic losses were estimated as 1588 LE =288$ for every unsuccessful service. Moreover, such losses become greater if the animal did not get pregnant before the end of breeding season.

4- DISCUSSION

Repeat breeding is among reproductive disorders which hinder favorable productivity in buffaloes [15].

In the present study, the incidence of typical repeat breeders was 4.34% of the total examined animals and 7.25 % of all cases having reproductive disorders (813). The same result was found by [3]. Meanwhile, [16] and [17] reported a range of 8.33 - 28% for this syndrome in bovines. Variations in incidences may be attributed to the heterogeneity or multifactorial causes of the repeat-breeder syndrome as well as the effect of locality, season and year [18].

The low progesterone level that was recorded in the current study in repeat breeder buffaloes during the luteal phase was similar to the result of [19] that attributed the failure of conception in these animals to their low progesterone level. Moreover, [20] indicated that RB heifers revealed higher P4 concentrations during estrus and early metoestrus, and lower P4 concentrations during late metoestrus and onwards. In this respect, [21] suggested that the supra basal level of P4 during estrus reduced tubal contractility and delayed sperm transport to the site of fertilization and early embryonic mortality. Also, [17] mentioned that the disturbed hormone level which prolonged standing estrus and delayed ovulation causes changes in the microenvironment of the preovulatory follicle, negatively affecting the final maturation of the oocyte leading to fertilization failure in those repeat-breeder heifers. In another study, he reported a negative correlation between conception rate and skim milk progesterone level in cows artificially inseminated [22].

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 overabundance of ROS, oxidative stress (OS) occurs. Also, ROS have a role in pathological processes involving the female reproductive tract, whereas, it affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy [23]. This theory was confirmed in the current study where RB buffalo-cows showed increased MDA and NO and decreased CAT, SOD , ASCA, GSH-R and TAC. An endogenous NO system exists in the fallopian tubes [24].

NO has a relaxing effect on smooth muscles and it has similar effects on tubular contractility. Abnormal concentration of NO may lead to tubal motility dysfunction, resulting in retention of the ovum, delayed sperm transport and infertility. On the other hand, it was reported that increased NO levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa [24]. Moreover, [25] found that NO inhibits ovarian steroidogenesis. The presence of endothelial NO synthase in corpora lutea and its expression has been reported in the mid and early luteal phase and to a lesser extent in the late luteal phase Moreover, [26] and [27] added that NO inhibits steroidogenesis in the corpus luteum and has luteolytic action mediated through increased prostaglandins and by apoptosis.

SOD is present in the ovarian tissue and it was found that there is a correlation between SOD and Ad4BP which is a steroidogenic transcription factor that induces transcription of the steroidogenic P450 enzyme. Thus, it controls steroidogenesis in the ovaries. The correlation between Ad4BP and SOD expression suggests an association between OS and ovarian steroidogeneis [28]. The preovulatory follicle has a potent antioxidant defense, which is depleted by the intense peroxidation [29].

Glutathione peroxidase may also maintain low levels of hydroperoxides inside the follicle and thus play an important role in gametogenesis and fertilization [30]. Meanwhile, [31] reported that glutathione is present in the oocyte and tubal fluid and has a role in improving the development of the zygote beyond the 2-cell block to the morula or the blastocyst stage

Vitamin C is a chain breaking antioxidant that stops the propagation of the peroxidative process and helps to recycle oxidized vitamin E and glutathione [32].

Increase in TAC was seen in follicular fluid of oocytes that later were successfully fertilized. Therefore, lower TAC is predictive of decreased fertilization potential [33].

The low concentrations of zinc, copper, iron and selenium traced in this study coincide with [34] and [35] who recorded that serum zinc and copper were significantly low in repeat breeders if compared to normal buffalo cows and added that when these animals were supplemented with 500 ppm zinc acetate in the drinking water and sodium phosphate 40 g/head/day in the diet for 1 month, respectively , the conception rate improved by 80%.and this explains our findings where the treatment with mineral supplementation gave the best results for conception (63.64 %) . This is in agreement with [15] who reported that 64.6 % of repeat breeder buffaloes came to estrus and 58.4 % conceived within one month after supplementation with vitamin/mineral mixture for 3 weeks. Then, he added that the hormone treatment is more effective than 3 weeks supplementation with vitamin/ mineral mixture.

Use of hormonal treatments such as GnRH or hCG, have been used by many investigators to increase the rate of pregnancy for repeat breeder cows [36, 37, 38]. It is suggested that it has a role in the expression of SOD as it is found that the Cu-Zn SOD expression in the corpora lutea paralleled with levels of progesterone and these levels rose from early to the mid luteal phase and decreased during the regression of the corpus luteum. However, in the corpus luteum from pregnant cases, the mRNA expression for Cu-Zn superoxide dismutase was significantly higher than that in midcycle corpora lutea [28]. Other investigators reported that when 36.4- 50.0 % repeat breeder buffaloes washed by 1 liter of 1% Lugol's solution conceived within one month after treatment [15].

From the economic point of view, the repeat breeding syndrome impacts the buffalo industry as it causes increased culling, reduced milk production, and reduced value of breeding stock. On the other side, the indirect costs of sound diagnosis, treatment trials, repeated artificial insemination should also be considered. RBS return the animal to service, increased time to conception and thus increased calving interval in the long-term reduced milk production or permanent infertility. The profitability of milking buffalo-cow increases with age, and culling earlier than the fourth lactation may result in net cost.Also reduced fertility is the commonest reason for culling in the UK [39], so any disease or syndrome affects fertility will have an economic impact. RBS may negatively affect milk production. Whilst an increased calving interval would reduce the number of lactations within a period of years, an RB may increase or prolong the lactation in which it happens. Thus, the impact of RB on milk production is complex and has not been fully quantified. In twenty-two Michigan dairy herds, repeat-breeder syndrome was observed in 24% of 3,309 lactations. Cost components associated with unsuccessful inseminations included costs of delayed conception, extra inseminations, extra veterinary service and losses due to culling. Lactations with repeat-breeder syndrome were associated with a loss of approximately $385. An estimated extra cost of $140 was associated with a second insemination, $279 with three inseminations, $429 with four inseminations and $612 with five inseminations [40].

It was concluded that RBS has economic impact on buffalo production and consequently, local meat and milk production in Egypt. Veterinary supervision should provide better animal health care and education to farmers regarding the risk factors that may lead to RB. Also, use of ultrasonography may help to get rapid and sound diagnosis. Great efforts should be done to catch up the breeding season not to lose the proposed new individual and lactation season. Also special care should be paid for minerals and food additive in animal's food stuff for animal welfare and breeder income.

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