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PREVALENCE, MAJOR RISK FACTORS AND RESPONSE TO HORMONAL THERAPY OF PHANTOM COW SYNDROME IN URBAN/PRERI-URBAN DAIRY FARMS IN BISHOFTU TOWN

BY:

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JUNE, 2023

BISHOFTU, ETHIOPIA

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A thesis submitted to Addis Ababa University, College of Veterinary Medicine and Agriculture impartial fulfillment of the requirements for the Degree of Master of Science in Veterinary Obstetrics and Gynecology (VOG)

MSc in Veterinary Obstetrics and Gynecology

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Prevalence, major risk factors and response to hormonal therapy of phantom cow syndrome

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DEDICATION

I dedicate this thesis manuscript to my heroic and generous elder brother **EDESA REGASA**, who passed away by leaving his eternal reminiscence to us when I joined the school for his ambition to get his talent through his brother and to my astonishing mother Bedatu Dibaba, who laid the foundation with strong pillars in my life, and my generous father Regasa Gelan for their endless love, support and most importantly, inspiration.

STATEMENT OF THE AUTHOR

I attest that this thesis is entirely my own effort. I prepared, collected, analyzed, and brought together the thesis by the ethical and technical standards of scholarship. Every piece of academic research that is mentioned in the thesis has been acknowledged or cited. I declare that this thesis is my bonafide work and the first in Ethiopia, Africa not authorized by any other researchers that all material sources used for this thesis have been duly recognized.

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Name: Onneta Regasa Gelan Signature: _______________

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LISTS OF ABREVATIONS

AI	Artificial insemination
BCS	Body condition score
CCI	Calving to conception interval
CI	Calving interval
CL	Corpus luteum
CR	Conception rate
DALC	Duration after last calving
DALI	Duration after last insemination
DMI	Dry Matter Intake
DZARC	Debre zeit agriculture research center
GnRH	Growth releasing hormone
HD	Heat detection
LH	Luteinizing hormone
NRNP	Non-return non-pregnant
NSC	Number of services per conception
OR	Odd ratio
PCS	Phantom cow syndrome
PGF2α	Prostaglandin F2α
PR	Pregnancy rate
RFM	Retained fetal membrane

ABSTRACT

Phantom cows that are non-pregnant and not returning to estrus within 21-24 days post insemination cause a serious reproductive management challenge for the smallholder and huge economic loss for the dairy producer. A clinical survey and therapeutic experimental study were conducted to determine the prevalence of phantom cow syndrome, determination risk factors and possibility of treatment using reproductive hormones in 25 dairy farms in and around Bishoftu town. Relevant data were collected through clinical evaluation of animals, record analysis reproductive indices and experimentation. The overall incidence of phantom cow syndrome was 11.9%; (86/720). Proportional incidences in small, medium and large farms were 47.5%, 11.8% and 5.8%, respectively. There was a significant difference ($P < 0.05$) between the different farm sizes. Presence of diseases such as Pyometra, metritis, luteal cyst, and cow related factors such as daily milk yield, age, parity, and body condition were found to be the major risk factors of PCS. There was a significant difference ($P<0.05$) in the incidence of PCS with respect to age, herd size, and parity. Return to estrus and pregnancy were highly improved in treated phantom cows with relatively higher pregnancy rate (75%) observed when cows were treated with PGF2 alpha and uterine lavage when there is endometritis. Early pregnancy diagnosis (between days 17 and 24) either with ultrasound or non-pregnancy test using a rapid P4Gold test was instrumental in restoring cyclicity in phantom cows. The prevalence of PCS in this study is relatively high; showing more non-productive animals are being kept in the dairy system. Many of the risk factors identified in this study are more common in the small holder dairy farms thus need to be considered during optimizing the production system. The use of a more accurate method of early pregnancy detection and treatment of phantom cows with reproductive hormones can contribute to the reduction of incidence of PCS by increasing submission rate for cows that fail to be pregnant during the preceding service. Therefore, proper diagnosis of phantom cows and their early treatment using hormones can potentially reduce the overall impact of PCS

Keywords: Anoestrus, luteal cyst, phantom cow syndrome, pyometra, ultrasound

1. INTRODUCTION

Ethiopia is Africa's largest cattle producer country, with 70.3 million cattle in 2020 (CSA, 2020a), but the country remains in the list of underdeveloped country with this livestock population. The country's milk production is currently very low causing serious concern in the livestock contribution in agricultural sector because livestock is the backbone agriculture and basis for the livelihood of millions of Ethiopians. Nevertheless, despite the fact that dairy cattle breeding in Ethiopia is supported by modern technology, shortcomings such as poor nutrition, poor management, lack of training and awareness of producer, lack of technical support from government organs, and rampant diseases have had a major impact on the productivity of dairy cattle breeding (Ismail, 2015). It would remain mystery to be poor with these livestock resources and popular indigenous breed of cattle in the era of technology generation.

In Ethiopia, dairy cows are vulnerable to a variety of diseases and disorders that have a high degree of impact on the animal's overall productive and reproductive performance (Tegegne *et al*., 2004; Lijalem *et al*., 2015), cause a huge challenge to dairy producer. Infertility in dairy cows is mainly caused by diseases such as deficiency diseases, infectious diseases, mastitis, ovarian cysts, and disorders with associated consequences such as endometritis and pyometra are common. In recent decades major risk factors such as cow health-related, environmental-related, and management-related conditions that affect the productivity of the cows are known to decrease the fertility of the cows (Walsh *et al*., 2011).

Among the various disorders and diseases that affect dairy cows, phantom cow syndrome (PCS) is one of the specific situations that affect the dairy industry at any level of dairy production systems. PCS is a subfertility condition that occurs when a cow has had one insemination and does not return to heat within 24-35 days but determined to be not pregnant by ultrasound scan or by rectal palpation (Nation *et al*., 2001; Cuttance and Mason, 2015). This main detrimental situation affects the reproductive performance of dairy cattle in the newly emerging small-holder dairy farms.

In developing countries, it results in a significant economic and reproductive waste, which can lead economic crisis and the end of the dairy industry due to reduced lactation length brought by delayed conception, low calf crop for replacement heifers, and a higher culling rate.

Despite the fact that different major studies on dairy cattle reproductive problems have been conducted, none on the condition known as "phantom cow syndrome" in Ethiopia have been completed. A major study of reproductive performance in Ethiopian dairy herds did not address the prevalence and incidence of Phantom Cow Syndrome in smallholder and modern dairy farms and the response to hormonal therapy of Phantom Cows. A preliminary assessment of the incidence of phantom cow syndrome by Amare, (2017) revealed 7.5% (25/334) prevalence. In traditional reproductive management, a phantom cow is not detected until pregnancy examination 40 to 60 days post AI (Nation *et al*., 2001). Such cows cause economic loss since they are neither lactating nor pregnant, and live only at the expense of other potentially reproductive and productive cows. Although phantom cows continue to create a serious reproductive challenge, there are still no other scientific studies carried out on the effects of PCS and success of hormonal therapy. The causes of PCS seem to be multifactorial spanning form failure of fertilization to early embryonic loss and various management holes. Therefore, it is hypothesized that the magnitude of PCS is determined by the level of reproductive health problems that derange hormonal balance and production related risk factors. Further, early detection of reproductive disorders through appropriate management and installing hormonal therapy can bring back cows into cyclicity potentially reducing the impact of PCS on the dairy system.

OBJECTIVES:

- To determine the prevalence of Phantom cow syndrome among crossbred dairy cattle in and around Bishoftu town.
- To investigate the risk factors contributing to a high incidence of phantom cows in dairy cattle in and around Bishoftu town.
- To evaluate the response of phantom cows to hormonal therapy

2. LITERATURE REVIEW

2.1. The Estrus cycle of dairy cattle

The estrus cycle of the animals refers to the narrow window (18-24hr) of time right at standing heat (estrus) and ovulation (commonly occurs about 12 hours after standing heat/estrus) for possible fertilization, and defined as the rhythmic physiological changes that occur in the reproductive system of a female animal controlled by neuro-endocrine action and last from 6 to 30 hr. Oestrous cycle is the continual cyclic changes of ovarian activity in all non-primate animals during the reproductive phase of an animal except the time of pre puberty, pregnancy, and reproductive senescence (Sing *et al*., 2021; Boer *et al*., 2011). It related to behaviors associated with estrous behaviors or estrous cycle and controlled by different reproductive hormones. The hypothalamus pituitary gonadal axis regulates both the estrus and oestrous cycle by producing hormones that control reproductive processes (Perry, 2004; NADIS, 2016).

Bovine females have generally two reproductive phases and four stage of oestrous cycles (Figure 1.); each cycle consists of a longer luteal phase (2-17 days) dominated by progesterone hormone, and a shorter follicular phase (18-21 days), which dominated by estrogen hormone. They begin at prepubertal heifers that have not had their first estrous cycles (Boer *et al*., 2011; Lamy *et al*., 2016), and cease at reproductive senescence depends on genetics, physiology and weight of the animals (Barcellos *et al*., 2014). The pattern and number of several hormones that are secreted regulates the growth and development of follicles and the corpus luteum (Lijalem *et al*., 2015; Bihon and Assefa, 2021).

Understanding the estrous cycle will improve your comprehension of reproductive management and regulate estrous cycle (Block *et al*., 2022), finally increase dairy owner profit. Controlling the estrous cycle can also boost the proportion of cows that conceive at the start of a breeding season (Perry, 2004; Boer *et al*., 2011; Barcellos *et al*., 2014).

The whole cycle and phase of estrus cycle with corresponding hormones are mentioned in (Figure 1).

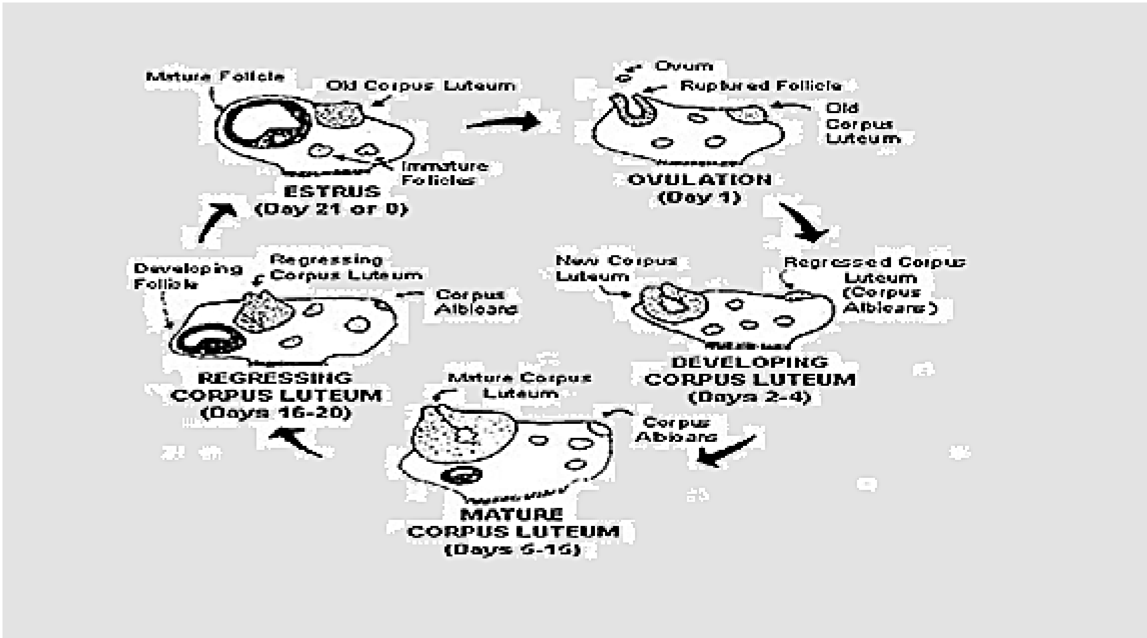


Figure 1: Schematic representation of estrus cycle and phase. *Source: Thomas 2015*

Estrus (day 0) is where the high estrogen concentration in the blood triggers a release of LH by positive feedback of GnRH near the onset of standing heat and lasts from 6 to 30 hours, with 20 hours being the average length. Luteinizing hormone (LH) spikes to a high-level during estrus by high threshold of estrogen hormone which influence positive feedback to the anterior pituitary result in ovulation (Larson, 2008; Lamy, 2016). Metestrus period (2-4 days) is the luteal phase, when CL is undergoing early development from the ovulated follicles and prevent the cyclicity (Yizengaw, 2017), and progesterone level in the circulation begins to rise, which has a negative feedback effect on the GnRH neurons in the hypothalamus if the animals became pregnant, restricting oestrogen production.

Diestrus (5-17days) phase of proliferation is the longest part of oestrous cycle, the period of maximum CL size and function, and high level of progesterone which prevents cyclicity by on anterior pituitary in a negative feedback fashion; then decreases the release of FSH and LH and prevents ovulation by inhibiting follicular development and maturation. Luteolysis of the matured corpus luteum is ceasing this period following the production and release of PGF2 alpha from uterus (Lamy *et al*., 2016), withdrawal the negative feedback mechanism of the progesterone. Proestrus (18-21days) is the secretary phase where the follicular phase starts and follicles begin developing by FSH and LF which in turn secrete oestrogen hormone. This produced high threshold level of estrogen from developed graafian follicles, causes positive influence on anterior pituitary in turn result surge of LH.

2.2. Estrus signs, Heat detection and Factor affecting heat detection

2.2.1. Behavioral sign of estrus

Normally, healthy, nonpregnant dairy cows and heifers should exhibit signs of estrous behavior (heat) every three weeks by successive ovarian function under the control of neuro-endocrine along the H-P-G axis. Estrogen is secreted by specialized endocrine cells located in tissues forming the walls of growing follicles and promotes both the external physical and visible behavioral signs of estrus by LH surge (Wiltbank and Cunha, 2008). Therefore, in the course of nature, females only become sexually driven and receptive close to the time when ovulation is about to occur by LH surge and conception is possible. No animal can exhibit evidence of ovarian activity through the initial growing period before the age of puberty, pregnancy, and at the age of reproductive senescence (Sign, 2021). At the time of puberty, animals can reach their optimum physical size to undergo successful conception and gestation and can show typical estrus behavior (Sign, 2020).

The pre-ovulatory follicles secrete a high amount of estradiol, which promotes a GnRH in turn play role in stimulating LH surge and leads to the expression of behavioral estrus by release of LH to cause ovulation (Yilma, 2020).

Standing heat is the primary estrus sign and can occur any time in a 24hour period based on the season of production. The other secondary sign of dairy animals includes; bellowing, swollen of vulva, clear watery vaginal discharge, restlessness, decrease milk production, sniffing another animal's vulva and flehming and curling lip (Sing *et al*., 2021). The animal is said to be in estrus when it stands to be mounted, demonstrating her acceptance of the male and her eagerness for sex (receptive to bull), sets estrus behavior like restlessness, decrease milk production, reddening of the labia, stand to be mounted for mating (Figure 2), and swelling vulva (Lamy *et al*., 2016), and lasts from 6 to 30 hours, with 20 hours being the average length (Lamy *et al*., 2016; Yizengaw, 2017).

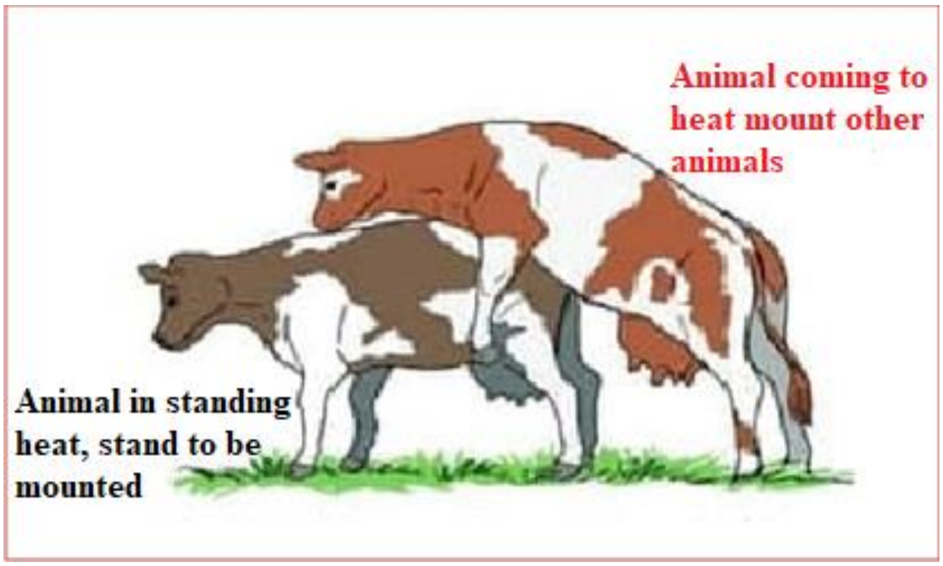


Figure 2: Schematic representation of cow in heat and coming to heat; *source: Wangare, 2021.*

2.2.2. Heat detection and Factor affecting heat detection

Estrus detection is one of the most critical steps and play pivotal role in dairy production and the most important factors affecting the outcome efficiency in dairy cattle reproduction, reproductive technology, primarly in the success of AI (Reith and Hoy, 2018; Sing *et al*., 2021). Monitoring cows and heifers as closely as possible, both early in the morning and late at night as well as in the middle of the day, are crucial to standing heat detection (typically lasts for 15 hours) (Chambers *et al*., 2020), and in turn increase the reproduction efficiency. The ability to determine standing heat is the product of heat detector experience, animal management, individual animal and environment. Having sufficient knowledge on the animal behavior during the breeding, knowing estrus sign of animals and heat detection is the crucial point in animal management to enhance the efficiency of dairy sector production and reproduction.

The specific time of the estrus sign or standing heat can occur in a 24hr period and affected by a season and stage of production, management, individual animal, and detector experience. It occurs mostly at night in hot weather and more exhibiting heat during the day in cold weather due to the infusion of estradiol that stimulates the neuron system highly affected by animal body temperature or heat (Jemal and Lemma 2015). Estrous behavior can be categorized as primary (mostly standing to be mounted) and secondary signs (other signs of estrus rather than standing to be mounted) (Yilma, 2020).

Moreover, Primary sign is the most typical and conclusive sign that an animal is in standing heat/estrus and ready to inseminate, whereas secondary sign alone is not the indicative of standing heat in dairy cows and it indicate whether the cow is coming to heat or going away from heat. Detecting animals in standing estrus is a significant to the success of any artificial insemination program and determining the exact time of cow at heat is an art (Jemal and Lemma 2015). Feasible detection of estrus is a permanent challenge for successful reproductive performance in dairy cattle and can be carried out by visual observation and with aids (Yizengaw, 2017).

2.3. Major factors affecting conception rate in dairy cattle

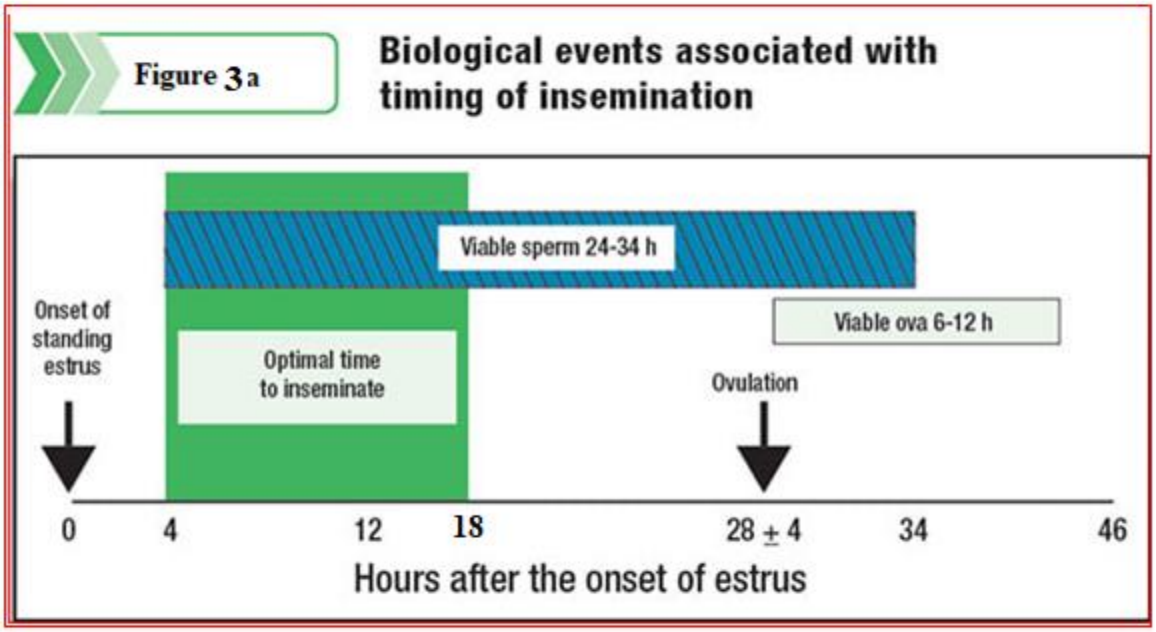
A dairy cow must give birth to one calf every 14months in averages and approximately five calves over the course of her lifetime in order to remain profitable. In Ethiopia, the dairy sector is subsistence-constrained by different mechanisms that reduce reproduction performance due to managemental, environmental, and other factors (Getabalew *et al.,* 2019). For profitable dairy cattle, two services per conception are still acceptable, but values above three indicate serious organizational and/or health issues with reproduction (Howlader *et al*., 2019).

Conception rates are influenced by a diversity of factors. According to Howlader *et al*., (2019) report, managemental and environmental variables share 96% of the variation in conception rates. Significant variations in conception rates may result from herd variances in diet, metabolic diseases, reproductive health, heat detection, insemination techniques, and climate.

2.3.1. Environmental and managemental related factors

According to De Rensis and Scaramuzzi (2003), summertime conception rates can drop by 20% to 30%, with clear seasonal patterns of estrus detection. High environmental temperatures impair the cow's capacity to engage in natural mating activity because they reduce both the duration and intensity of estrous expression (De Rensis and Scaramuzzi, 2003). It has been proposed that decreased DMI and its associated effects on hormone synthesis cause a decrease in estrous behaviour (Chambers *et al*., 2020). Moreover, dairy cattle breeds, which have attempted to change the cow from a seasonal to year-round breeder, may be to be blamed for reduced estrous behavior (Mellado *et al.*, 2014). A number of researches indicate that high temperature has a negative effect on the reproduction process of dairy cows owing to undetected estrous cycle, decreased estrous period intensity effects on physiology and decline fertility (López-Gatius, 2013). The survival and mobility of sex-sorted sperm, as well as the survival and motility of embryos, are all adversely affected by high environmental temperature (Mellado *et al.*, 2014).

Management variation can be categorized into intrinsic and extrinsic variation. Heat detection techniques, use of skilled inseminator, right place of insemination relative to female genital tract, right time of insemination relative to estrus to ovulation are considered as factor for successful conception (Filho *et al*., 2010). The ideal timing for insemination to occur relative to ovulation (insemination-ovulation interval) is mostly determined by the viable lifespan and the 24- to 36-hour fertile lifespan of spermatozoa (24-36 hour) and on the viable lifespan of the oocyte (Figure 3a) in the female genital tract (6-12 hours). For conception to occur, insemination must take place at the correct stage of the cow's estrus cycle (Figure.3a and b) since ova remains viable for about 12-18 hrs. after ovulation (Roelofs *et al*., 2005).



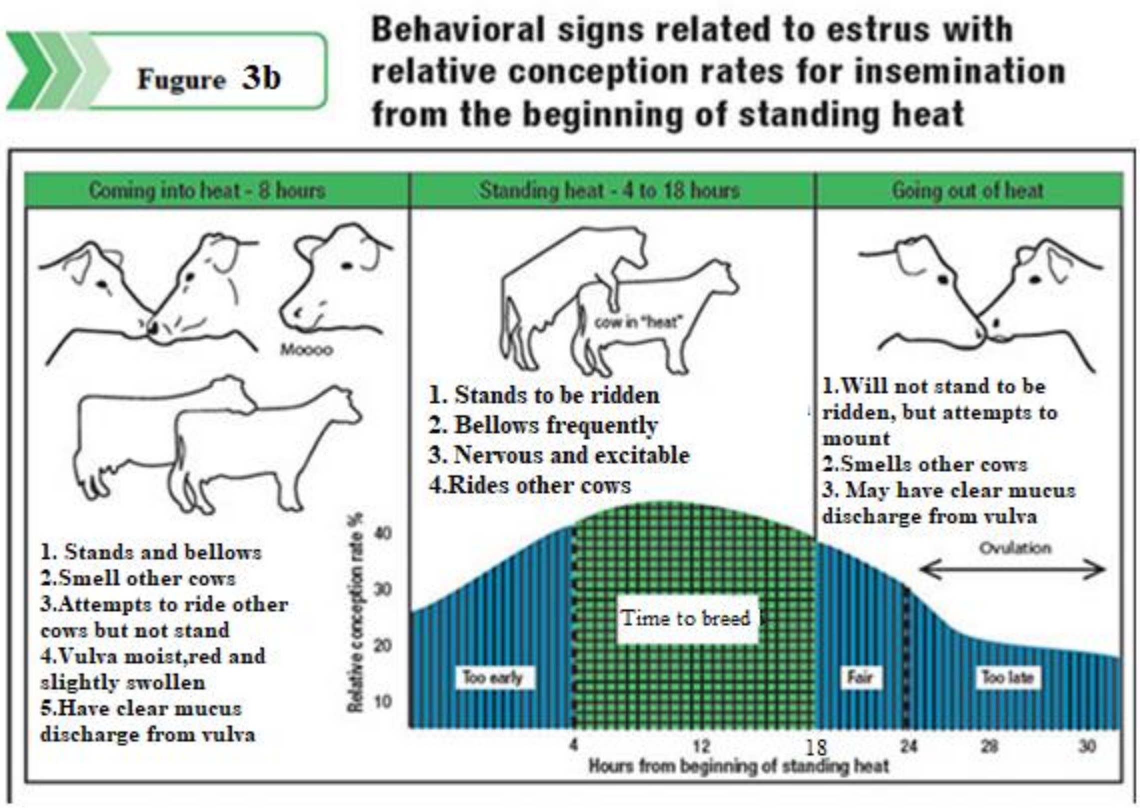


Figure 3: Schematic representation of the AI effective time interval (a) and biological changes in cows (b); *Source: Nebel, 2014*

Dairy cows losing their body condition, inevitably prone to deficiency of macro and micro minerals of which micro minerals have more tricky effect on fertility of dairy cows. Nutritional infertility associated with hypoglycemia has been recognized in cattle; lack of phosphorus or Ca: P imbalance may reduce fertility in cattle (Xu *et al*., 2010). Vitamins and trace elements are in general important in steroid hormone synthesis, expression of growth factors and gene transcription which is required for embryo cell proliferation and differentiation. Deficiencies of vitamin A cause failure of embryonic growth and disruption of organogenesis while it's excess also cause teratogenesis (Lonergan *et al*., 2016). Vitamin E and selenium are antioxidants and their deficiency also lead to embryo loss at time of implantation. Cobalt interferes in folate production and its deficiency also impairing to embryonic development (Xu *et al*., 2010).

2.3.2. AI Kit Hygiene and Semen Handling

A stainless-steel artificial insemination gun, straw cutter or scissors, tweezers (forceps), non-spermicidal lubricant, thermometer, wide-mouth thaw thermos, and disposable items like split plastic sheaths, sanitary covers, plastic palpation gloves, and paper towels must be kept in a dry, dust-free, and clean place before and after artificial insemination. Straw extraction should be done below the lower half of the neck of the storage tank, by keeping the canister, cane, and unused straws as close to the neck of the tank as possible for no longer than 5-8 seconds, where the temperature is sufficiently cold (-120ºC), in order to avoid exposure to high temperature (promotes thermal damage) (Cortés-Beltrán *et al*., 2021). Efficiency in handling is essential to reduce exposure time outside of the tank and preserve the quality of the frozen semen in order to prevent this risk. To avoid thermal damage to the semen and potential operator harm, it is crucial to handle the straws with tweezers rather than your fingers.

2.3.3. Postpartum reproduction disorder

The Postpartum uterine diseases are negatively associated to the reproductive performance of cows (Ribeiro *et al*., 2013) and lead economic crisis to the producer. The postpartum uterine diseases (Dystocia, retained placenta, clinical metritis, clinical endometritis, subclinical endometritis, and others) result from structural and/or functional damage to the reproductive organs and significantly decrease fertility in extensive and intensive dairy production system (Bonneville-Hébert *et al*., 2011; Vallejo *et al*., 2020). According to Bonneville-Hébert *et al*. (2011) report, the first service and conception rate of animals exposed to clinical ketosis, dystocia and retained placenta were delayed about 13 days and lower than 4 to 10% respectively and require 6 to 12 more days to conception. Metritis was resulting in 19 more days to conception rate. Cystic ovaries and anestrus following to calving were associated with 11 more days to first service and with 30 more days to conception, resulting in 41 more days to conception and 70 to 80 more days to conception respectively (Fourichon *et al*., 2000).

2.4. Phantom cow syndrome

Phantom cow syndrome (PCS) is disease condition that represents non-cycling non-pregnant animals that cause a significant loss in reproductive performance of the dairy herd and hence influence productivity. Phantom cow syndrome is sub fertility condition mostly defined as cows that have a long oestrous cycle return after insemination (Nation *et al*., 2001), i.e. not detected in oestrus 25 to 45 days after insemination while not pregnant and sometimes even more than this range though some controversies on time range of a cow turn to the next estrus (Cuttance and Mason, 2015).

Phantom cows are cows that are artificially inseminated early in the breeding period, not detected in oestrus within at least 24 days of insemination, and later classified as having failed to become pregnant at the first insemination when retrospectively diagnosed (Cavalieri *et al*. 2003b). It is the percentage of cows that weren't re-submitted for a second insemination at least 24 days after being retroactively determined to be not pregnant (Cavalieri *et al*. 2003a). PCS is a major cause of subfertility in Indian, New Zealand, and Australian dairy herds (Nation *et al*., 2001) and hinders the economy of new dairy farms.

Phantom cow syndrome impacts the economy of the farm by wasting the reproductive performance of the animals for a long period, decreasing the production and reproduction of the individual animal, decreasing the lactation period of the cow's lifespan, and extending CCI and CI. According to the Nation *et al. (2001)* report, there was a significant association between Phantom cow syndrome and the physiological state of the animals before the first service and nutritional management during transitional period (Cavalieri *et al., 2003b*; Chambers *et al*., 2020).

2.5. Risk Factors of Phantom Cow Syndrome

Roughly one third of dairy cows culled annually in the Australia, New Zealand, and India are due to reproductive problems like phantom cow syndrome and other conditions (Nation *et al*., 2001; Wu *et al*., 2012; Ott *et al*., 2018). For low output late in lactation, lactating cows that are neither detected in oestrus within at least 24 days of insemination nor conceive (phantom cows) are eventually culled at a considerable cost to the dairy farmer (Ott *et al.*, 2018). The major risk factors that have the largest contribution towards Phantom Cows (NRNP cows) syndrome are categorized into animal related factors and managemental related factors:

2.5.1. Animal reproductive-related factors

Anoestrus: It is a functional disorder of the normal cyclical order of reproductive cycle. Physiological and pathological anoestrus is the major anestrus in dairy cattle. But there are different types of anoestrus conditions manifested by dairy cattle. It includes postpartum anestrus (true anoestrus) which is characterized by the absence of estrous behavior, which may be an indication of suboptimal condition (e.g., inadequate peripartum nutrition) or pathologic condition like uterine and ovarian diseases. The other type of anoestrus is lactational anoestrus which is due to production stress (NADIS, 2016). Nutritional anoestrus is also observed in early postpartum period or lactating dairy cows (Kumar *et al*., 2014) and types of anoestrus in cattle are reported.

Heritability: The heritability of high producing dairy cows of estrus expression changes greatly from one estrus period to the next. The ability of the cow to cycle again and go into heat following calving and the ability of the cow to conceive after insemination and become pregnant were two aspects of fertility traits in dairy cows (Aungier *et al*., 2015). According to different researcher report, the time between calving and the first insemination (CFI) relation to the interval between calving and the first high activity (CFHA), estrus detection length (EDL), and estrus strength (ES) are affecting cow fertility (Holstein) (Ismael *et al*., 2015).

For instance, Jersey cows and heifers have more intense and longer periods of estrus expression as compared to Holstein cattle (shy in estrus expression and mostly categorized under short and low to medium estrus duration and intensity, respectively).

Healthy related: Modern dairy production relies heavily on early successful conception of postpartum dairy cows to achieve the highest levels of reproductive efficiency and profitability. Cows with mastitis, retained placenta, metritis, lameness, or digestive diseases (Ribeiro *et al*., 2013), have delayed conception and reduced fertility (Gebreyohanes *et al*., 2021). Decreases in fertility are seen regardless of whether these diseases are uterine-related or not and have long-lasting effects on reproduction (Carvalho, *et al*., 2019). Increased pregnancy losses lead to longer gestational periods, higher risk of culling, and lower milk production during lactation. Fertility declines have been linked to everything from the survival of the developing foetus to the fertilization of the oocyte or egg (Bisinotto, *et al*., 2018; Xu, *et al*., 2020).

Age and Parity: The incidences of PCS cows are most likely to occur in heifers and primiparous cows (milk stress and clearance of progesterone hormone), followed by older and multiparous cows, and are least likely to occur in middle aged cows. Although this association has not been proven in older cows, the greater incidence of NRNP cases in 2-year-old cows (primiparous) may be related to their increased risk of being anestrus (Nation *et al.*, 2001a). Chambers (2020) report, the increasing prevalence of PCS instances in older cows may be related to higher rates of embryo death or impaired luteal function.

Postpartum disorders: Problems during the calving process affect the performance of postpartum physiological and overall biological processes of cows. Any interference during the calving process such as a dystocia, retained placenta, uterine and vaginal prolapse, and other ill conditions will most likely cause delays in the return to cyclicity and induce uterine infection. Reproductive disorders occurred at calving and postpartum are more complex and impact on the animal’s life span fertility and cows with reproductive disorders have longer intervals from first service to conception and required more services per conception (Shiferaw *et al*., 2005).

Cystic ovarian disease (COD): It is pathological ovarian cysts which arise as the result of anovulation of follicles. Follicular cysts may be single or multiple on one or both ovaries tend to be thin-walled. Luteal cysts are thicker walled follicular structures that have undergone partial luteinization without the occurrence of ovulation and appear usually as a single structure (Nelson *et al*., 2010) while luteal cysts are less frequent than follicular cysts and both are pathological (Cattaneo *et al*., 2014). Cystic cows exhibited lower conception rates and longer calving to first service and calving to conception intervals than controls (Cattaneo *et al*., 2014), due to the hypothalamic-pituitary-ovarian axis an endocrine imbalance associated with an absent, insufficient and/or asynchronous luteinizing hormone (LH) surge (Nelson *et al*., 2010).

According to the report of Nelson and his colleague, some factors predisposing to COD are heredity, dystocia, ketosis, milk fever, high milk production, age, lactation period, body condition score and phyto-oestrogens (Cattaneo *et al*., 2014). Retained placenta, milk fever and metritis are mentioned as possible factors associated with the prevalence of the disease (Nelson *et al*., 2010). Finally, it has been proposed that stress is a key cause of COD because it prevents the oestrogen-induced LH surge by ACTH.

Fertilization and implantation failure: a) Fertilization Failures- Potential conceptions could be missed due to some type of fertilization failures or ovulation disturbances. These are caused by many factors including lost eggs, ovulation failure, blocked oviducts, breeding too soon or too late relative to the onset of estrus, abnormal eggs, low fertility, and poor-quality semen. In general, aged cows have lower fertilization rates than younger cows. In heifers, fertilization rates may approach 100% (Lonergan *et al*., 2016). Fertilization failure occurs due to delayed ovulation, lesions of the oviduct, thermal stress, poor accuracy of heat detection and ovarian cysts (Santos *et al*., 2004).

b) Failure of maternal recognition pregnancy (MRP): MRP refers to the process by which the conceptus signals its presence to its dam and must take place about day 17 or 18 (Bilodeau-Goeseels *et al.*, 2003).

The stage is critical since the embryo must be developed sufficiently to override the spontaneous uterine secretion of PGF2α by secreting bovine interferon-tau (bIFN-τ). Size of conceptus also matters in effective blocking of PGF2α secretion: smaller conceptuses that are apparently developmentally delayed may not secrete enough bIFN-τ to block luteolysis (Bilodeau-Goeseels *et al*., 2003). Secretion of various substances (proteins) by the developing embryo and its rudimentary placenta (trophoblast) are involved in maintaining the successful continuation of pregnancy. Then conceptus occupies most of the uterine horn ipsilateral to the CL. PGF2α is normally causes the corpus luteum to die to initiate another period of heat and a new estrous cycle. Losses at this stage often are detected because the cow generally return to estrus after an extended period (>24 days or longer than a normal estrous cycle) (Bilodeau-Goeseels *et al*., 2003).

Embryonic losses: Embryonic mortality in cattle is categorized into two phases; early embryonic losses (losses of conception prior to day 24), and late embryonic losses (after days 24 to 50) (Santos *et al*., 2004; Kinne, 2016). While pregnancy losses detected after day 50 characterize as fetal losses (Santos *et al*., 2004). It could be starting from the embryo enters the uterus and when the morula is developing into the blastocyst, is where early embryo death (EED) often occurs. Around 25-30% of pregnancies fail due to early embryo mortality (Chambers *et al*., 2020). Embryonic/fetal loss occurs throughout pregnancy in cattle; however, it is concentrated mainly in the first 42 d after breeding, i.e., in the embryonic phase of pregnancy (from fertilization to completion of differentiation) (Bilodeau-Goeseels *et al*, 2003; Haile-Mariam *et al*., 2003; Cuttance and Mason, 2015).

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2.5.2. Managemental related factors/failure

Management factors can be altered by development of a consistent management programs for regulating reproduction, while changing physiological factors can prove difficulty. Often, separating the physiological and management factors at on farm situation is difficult (Haile-Mariam *et al*., 2003).

Heat detection failures: Heat detection (HD) failure exacerbates phantom cow syndrome in a herd since it means missed heat/ silent heat and extend CCI. Proper heat detection techniques, use of skilled inseminator, optimum time of insemination relative to estrus to ovulation are considered for successful conception (Filho *et al*., 2010) and the failures of this entire component can initiate PCS in dairy herds. The optimal time at which insemination should take place relative to ovulation (insemination-ovulation interval) depends mainly on the fertile lifespan of spermatozoa (24-30hrs.) and on the viable lifespan of the oocyte (12-18hrs.) in the female genital tract. For conception to occur, insemination must take place at the correct stage of the cow's estrus cycle since ova remains viable for about 12-18 hrs. after ovulation (Roelofs *et al*., 2005).

BCS: Due to its high correlation with animal feeding and healthy management, Phantom cows are most likely occurred in animals that have a BCS $\geq$4.5, compared to animals with a BCS < 4.5 in New Zealand and Australia dairy farms on BCS scale (1-9). It is of interest that cows that have a BCS >4.5, has higher incidence of NRNP compared to animals with a BCS< 4.5 (Nation *et al*., 2001); as animals with higher body condition score have poor reproductive performance, calving problem, BCS loss after calving and produce uterine infectious.

Nutritional deficiency: The Common nutrient deficiency that affects reproductive efficiency in dairy cattle includes Energy, protein, vitamins and minerals (Yami *et al*., 2013). Age at puberty, sexual development, and transitional phase are significantly impacted by energy deficit in dairy animals due to disturbance of growth and endocrine hormones, reduce production and reproduction performance. Protein deficiency/excess influences reproduction due to its impact on feed intake and can decrease the ovulation rate. Vitamin A and E are of great importance in maintaining cellular integrity and antioxidant property respectively and they also help in maintenance of immune status of animals which in turn straightly influences upon the health of the animal (Bindari *et al*., 2013). The major classical vitamin A deficiency includes delayed onset of puberty in males and females, failure of epithelial development, abortions and birth of weak, blind or deformed calves, increased incidence of retained placenta with keratinization, suppressed libido in males (Bindari *et al*., 2013).

Selenium deficiency is linked to Vitamin E deficiency. It reduces fertility by affecting fertilization and viability of spermatozoa, and increases the incidence of retention of placenta.
Copper (Cu) deficiency results in depressing the estrus sign by inhibiting the section of LH, Delayed ovulation and poor conception rates have been associated with Manganese (Mn) deficiency in cows. Zinc (Zn) deficiency affects the reproductive performance by preventing spermatogenesis and passively increases embryo mortality in male and female dairy animals respectively. In general, the effect of these nutritional deficiencies on reproductive performance has multi-stage and negative relationship especially decline the dairy reproduction by increasing early embryo mortality, delayed oestrous cycle, anostrus and silent estrus of the animals (Bindari *et al*., 2013). Optimum amount of nutrient should be recommended for dairy cows at proper time, physiological activity and based on other health condition of the animals.

Nutritional deficiency affects the normal cyclical order of oestrous cycle in different ways. Nutritional factors can promote or inhibit cyclic ovarian function. Severe mineral and protein deficiency interfere with reproductive processes in dairy cows. Total dietary intake can affect fertility, both at the level of the follicle growth, oocyte and embryo (Reith and Hoy, 2018). Energy restriction influences reproductive function through depression in gonadotropin releasing hormone (GnRH) in hypothalamic centers in the brain. Nutritional, metabolic and hormonal signals which may influence hypothalamic function and GnRH secretion include specific amino acids absorbed from the diet (e.g. tyrosine), glucose, ketone bodies, and insulin (Ferguson, 1996). By about 8 weeks after calving, cows have a positive energy balance as a result of the negative energy balance and fat mobilization stimulate DMI (Ferguson, 1996). On the contrary Wiltbank and Cunha (2008) reported that as compensatory, high yielding dairy cows require high dry matter intake and that intern affect reproductive cycle through the mechanism as shown in the following (Figure 1).

An altered follicular growth pattern might impair oocyte developmental competence. Biochemical parameters, associated with a negative energy status, are well reflected in the follicular fluid and can directly affect oocyte competence. Sutton-McDowall *et al*. (2014) reported that an adverse effect of a negative energy balance has direct impact on oocyte quality which is directly linked with endocrine environment.

Folliculogenesis is a very complex but finely tuned process, in which endocrine and paracrine signals play an important role (Orihuela, 2000; Wiltbank and Cunha, 2008; Leroy *et al*., 2008).

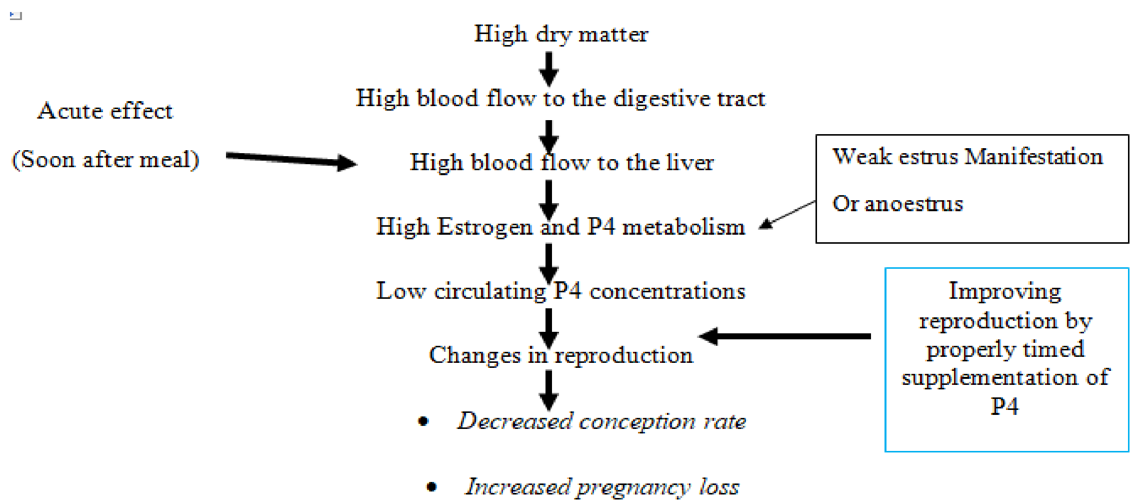


Figure 4: Effect of high dry matter intake on hormonal metabolism and reproduction. *Source: Wiltbank and Cunha (2008).*

Housing: Dairy cows housing is associated with an increased prevalence of several serious diseases including mastitis, delayed calving, retained fetal membrane, and lameness (Shepley and Vasseur, 2021; Hatew *et al*., 2023). For example, cattle kept in barns displayed more mounts per hour during oestrus than cattle kept in pastures. Estrus will be affected by the type of floor, unusual noise in the barn, low ceilings, deep mud, and frequent milking. Increasing stocking density of cows will increase the frequency of oestrous through easy meeting and interacting sexually. Dairy cows housing is associated with an increased prevalence of several serious diseases including mastitis, delayed calving, retained fetal membrane, and lameness (Shepley and Vasseur, 2021; Hatew *et al*., 2023).

Proper animal housing directly facilitates optimal health, production and welfare of dairy animals (Phillips, *et al*., 2013) Thermal humidity index (THI) under animal houses may exceed moderate and severe heat stress conditions. Dairy animals under extreme stress could have a significant decline in their physiological state (Singh *et al*., 2020).

The major classical general management related factors that affect dairy cows with phantom cow syndrome is improper manure management and waste disposal, slippery floor, lack of hygiene, lack of bed barn and space, dense herds, and lack of milking space are the major risk factors of PCS in dairy industries (Singh *et al*., 2020 - 2021).

2.6. Diagnosis, treatment and prevention of phantom cow syndrome

Inadequate/poor nutrition, stressful environments, large parasite burdens, and infectious diseases are the main risk factors for phantom cow syndrome (PCS). One of the main reasons dairy cows remained anoestrus during breeding season is due to phantom cow syndrome. Among the hidden causes of phantom cow syndrome are early embryonic mortality, endometritis, pyometra, mummified fetus, cystic ovary, milk production stress that causes hormonal imbalance, vitamin and mineral deficiencies brought on by a lack of fodder and feed, inadequate floor space, ventilation, and lighting (Nation *et al*., 2001; Singh, 2019).

The major clinical sign of phantom cow syndrome includes extending CI, increased DALC and DALI, increase or decrease BCS, anoestrus, and missing estrus, due to an issue with the animals' reproductive activity (NADIS, 2016). Cross-breed cows with Phantom cow Syndrome cause producers to lose a lot of profit (Singh, 2019). Observing the normal clinical exhibition of this illness in cross-bred cows can be confusing and difficult. Regular pregnancy checkups (rectal palpation and ultrasonography) and the detection of pregnancy-associated glycoprotein levels are two popular ways to diagnose the PCS. or the evaluation of hormones in milk samples in addition to the history and rectal examination of the animals.

Accordingly, based on the findings of the ultrasound examination and management enhancements, the treatment entails synchronizing oestrus, managing feed alternatively, managing the cattle shed alternatively, modifying or correcting the underlying disease condition, and dealing vitamin and mineral deficiencies (Singh, 2019). Generally, having record keeping of each herd, observation of inseminated cow and not come to heat after 25 days of insemination, pregnancy diagnosis through rectal palpation and cross confirmation of pregnancy throughout the first week of the second pregnant trimester is the major diagnosis method of phantom cow syndrome in dairy herds. The single most important anticipatory measure is to calve cows down to allow them sufficient time to recover before the next service season. Improve nutrition, mineral/vitamin supplementation, reducing heat stress, understanding the causes of anestrus and embryo mortality that increase the number of nonpregnant cows and remove it, and improving estrus detection and pregnancy checkup are among the preventative mechanism of phantom cow syndrome in dairy herds.

2.7. Current status of phantom cow syndrome (PCS) in Ethiopia

Although not modernly studied, PCS is occasionally seen in dairy cattle less than four years old of age, and farmers call the condition 'urda' (community), common in the predominantly Borana and Horro breeds. A preliminary assessment of the incidence of phantom cow syndrome (Kasa, 2017) revealed that the incidence of PCS in Holstein cross dairy cows was around 7.5% (25/334) in and around Bishoftu town. It includes the adoption of a planned mating system in which cows are synchronized for an initial oestrus. Several authors reported that feeding problems, anoestrus condition, postpartum disorders, poor body condition and overall management limitations are common in field as well as farm conditions of Ethiopian dairy production system (Lobago et al., 2005; Shiferaw *et al*., 2005; Mekonnen *et al*., 2010; Hunde *et al*., 2015; Jemal *et al*., 2015). In the presence of these all risk factors, the performance of dairy animals was wasted and the conception rate was delayed and the fertility efficiency is reduced sharply and consequently, the hidden phantom cow syndrome is inevitably prevalent in such dairy farms.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted from November 2022 to May 2023 at randomly selected dairy farms, in and around Bishoftu town. Bishoftu town located 47km southeast of Addis Ababa, at 9°N latitude and 4°E longitudes, and an altitude of 1850m.a.s. l (CSA, 2020a). The average minimum and maximum annual temperatures range from 10.550°C to 27.450°C, respectively, which makes the average annual temperature of 19.0°C. The average monthly relative humidity is 57.12% (DZARC Agro-Meteorology 2020). The town receives bimodal rainfall with two rainy seasons in a year. The short rainy season (15%) occurs between March and May and the main rainy season (85%) is during June to September. With these raining seasons, the average annual rainfall is 779.3mm. This site was purposively selected based on its suitability and accessibility of the study animals (dairy farms and cows), its representativeness in agro-ecology and dairy management.

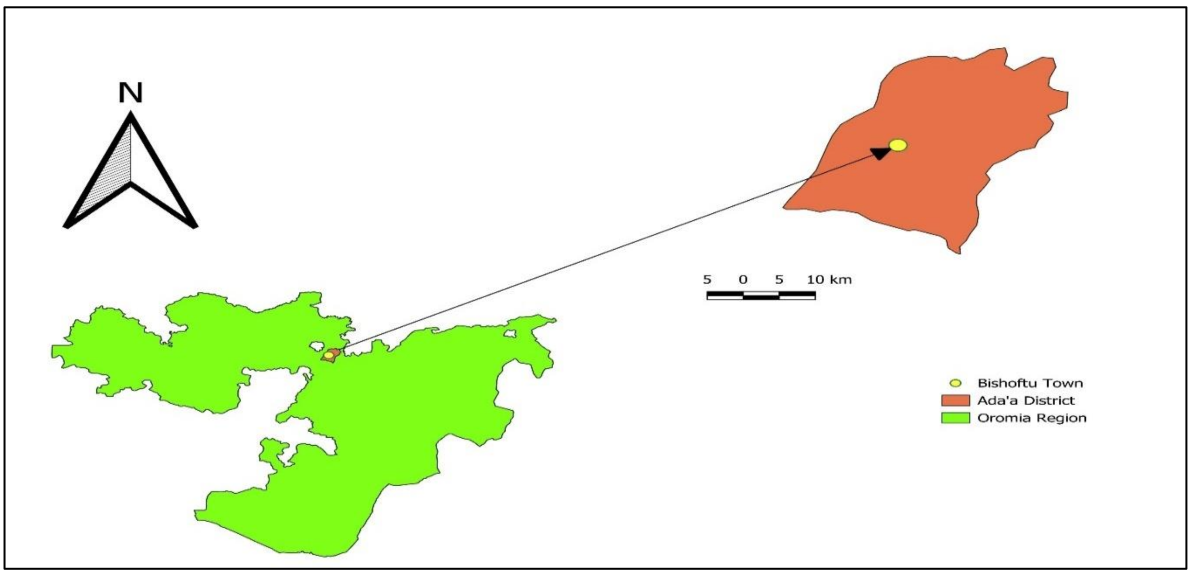


Figure 5: Geographical location map of study area (Bishoftu town); *Source:* Supported by QGIS

3.2. Study population and animals

The study animals are dairy cows in all small, medium, and large dairy farms in and around Bishoftu town. Holstein Friesian cross dairy animals with varying age, anestrus history for a long time, long DALC, DALI, CI, BCS, history of different reproductive disorder (dystocia, RFM, abortion/embryo loss, mastitis, uterine infection, lameness), parities, management, milk yield and lactation length for the prevalence of phantom cow syndrome. The sample size of dairy farms in the prevalence study was determined according to Arsham (2007) using the formula

$$N = 0.25/SE^2$$

Where n is the sample size & SE = Standard error of the population, approximately 0.1

Accordingly, 25 dairy farms consisting of 720 dairy animals were purposively selected and classified as small (n=10), medium (n=9), and large (n=6) dairy farms mainly based on the herd size and level of production (Megersa *et al*.,2011). The farms were selected considering the accessibility, willingness of the owners, and the availability of proper record keeping.

3.3. Study design and data collection

Clinical survey: All animals in the selected farms were clinically evaluated and their records reviewed to determine if they suffered from PCS. Animals that were found fitting to the definition of PCS were considered positive for the case. Further, various cow related factors and farm related conditions were also systematically evaluated and recorded. Age, presence of lameness, reproductive stage of the cows, lactation, parity, and presence of mastitis were examined and recorded. Feeding management, housing condition, and floor system were assessed and recorded. Records of reproductive disease such as retention of fetal membrane (RFM), dystocia, anestrus, history of abortion, and other reproductive health problems (uterine prolapse, pyometra, and metritis) were collected from farm register or/and owner's interview.

Experimental and follow up study: Animals identified as suffering from PCS in the previous study were randomly selected. A total of 38 cows confirmed to be phantom cows were selected and randomly assigned to three treatment groups: Group I (n=11), Group II (n=11) and Group III (n=16).

The groups were treated using IM 100µg GnRH (Receptal, Intervet international B.V., Netherland), 500µg of PGF2α (Synchromate®, cloprostenol sodium, Warburg, Germany) and a vaginal insert of Controlled Internal Drug Release (CIDR 1380, EAZI BREED™, New Zealand) by CIDR applicator at various combinations for each treatment group (Figure 6 to 8).

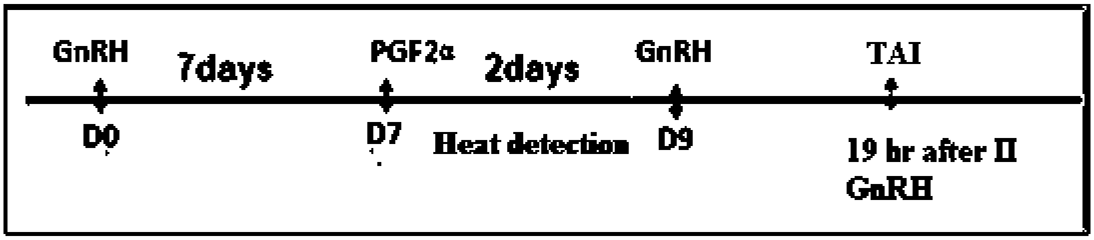


Figure 6: Group I (n=11): Ovsynch protocol

Figure 7: Group II (n=11): Ovsynch protocol with CIDR priming

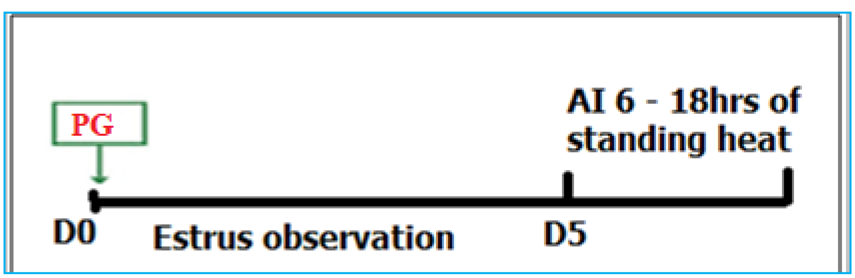


Figure 8: Group III (n=16); Single shot PGF2α protocol

Prior to the start of the experiment, the status of the reproductive organs (ovary, uterus, and cervix) of candidate animals was examined using ultrasound (with 5MHz B-Mode, linear array probe; EDAN DUS 60 Vet Ultrasound, USA). Additionally, body temperature was also taken at the time of examination. Animal with obvious clinical signs of reproductive diseases (eg pyometra and metritis) were treated first and became only eligible in the experiment after confirmation of commencement of ovarian cyclicity (Appendix 4). At the time of ultrasonic evaluation, records of ovarian structures (size and number of ovarian follicles, type and size of the CL, presence of cystic structures, shape and the presence of any abnormal condition in the uterus were assessed and lavage by saline water, povidone iodine, antibiotic with uterine douche and recorded on a predesigned data collection sheet.

Detection of estrus and AI of treated cows: The critical and routine activity was detection of estrus (standing heat) following to hormonal treatments closely monitored twice daily (morning and afternoon) for five days starting 24hrs following each treatment in all PGF2 alpha treatment groups. Artificial insemination of such treated groups was carried out accordingly by follow up observation of standing heat, and semen insemination was taking place 12–18 hours after standing heat for PGF2α treatment protocols (appendix 2 and 3). Insemination for (fixed time AI) FTAI groups of animals was conducted at 19 hours after the second GnRH injection on day 9 of the first GnRH injection.

3.4. Pregnancy diagnosis and identification of true phantom cows

All inseminated cows were diagnosed for pregnancy first by observation of return to service 17 to 24 days later to AI and then using P4 Gold test (Ridgeway Research Ridgeway Science Limited T/A P4golden, UK) and ultrasonography for confirmation of pregnancy (appendix 4). Cows that were neither pregnant nor returned to estrus were identified as true Phantom Cow.

$$\mathbf{CR} = \frac{\textbf{Number of conceived animals}}{\boldsymbol{Eligibe\ animals\ to\ conceive\ (animals\ in\ standing\ heat)}}; \ \mathbf{PR} = \frac{\textbf{Number of pregnant animals}}{\boldsymbol{number\ of\ conceived\ cows}}$$

3.5. Data management and statistical analysis

The collected data were grouped according to the age of animal, parity, anestrus, treatment group, and farm size and coded, and stored in a Microsoft Office Excel 2010 spread sheet. Descriptive analysis was used to determine the, frequency, percentage and proportion of different statistically non-significant data. A descriptive analysis was used to determine the proportions of animals that showed different patterns of uterine abnormal fluid accumulation, inactive ovary and anovulatory. The diameters of luteal cyst, the status of uterus (flaccid or tone) were recorded daily during the ultrasonography scan. In order to identify any significant causation, all binary data were performed by logistic regression by using STATA version 14.0 (Copyright 1985-2015 StataCorp LP, Texas, USA). Statistical significance of the study was considered at P value < 0.05 as significant. The effect of risk factors was described by OR and its 95% CI.

3.6. Ethical clearance

All activities were conducted out in compliance with the ethical guideline of the Addis Ababa University College of Veterinary Medicine and Agriculture after receiving the ethical approval (Ref. No: VM/ERC/04/15/023,15/02/2023; in appendix 5)

4. RESULT

4.1. Prevalence of Phantom Cow Syndrome and its major risk factors in dairy farms

According to the clinical survey, the prevalence of PCS was 11.9% (86/720; Table 1). The clinical survey showed a relatively high proportion of PCS among the small holder herd. Summary of PCS prevalence result in all dairy herd category is shown in (Table 1).

Table 1: Frequency of PCS prevalence among different herd size

Herd size	Total Herd	Animals with PCS	Prevalence (%)	P- value
Small (n= 10)	80	38	47.5	
Medium (n=9)	178	21	11.8	0.000
Large (n=6)	462	27	5.8	
Total (n= 25)	720	86	11.9	

Table 2 show the details of animals related risk factors that have a significant difference to PCS. The age-wise association was highly correlated to the incidence of PCS. Lactation length, milk yield per day (MYD), and herd size that were recorded by clinical survey were among the major risk factors to the incidence of PCS and statistically different at (p value<0.05) with respective 95% CI.

Table 2: Overall animal related associate risk factors (n=720)

Risk factors	No. of animals	Percentage (%)	OR	P-value	95%CI
Age					
< 3 years	57	66.5	2.5	0.000	[0.117 - 0.504]
>3 year	29	33.5	Refer.		[0.605 - 5.126]
Parity					
<3	67	78	68.45		[14.1- 34.02]
>3	19	22	Refer.		[0.13 – 0.54]
Milk yield					
<12L	74	86	6.14		[2.76 – 13.65]
>12L	12	14	Refer.		[0.01 – 0.07]
Lactation length					
6-9	27	31.4	Refer.		[0.237 - 1.304]
>9	59	68.6	2.3		[1.173 - 3.506]
Herd size					
Small	38	47.5	4.19		[1.58 – 11.1]
Medium	21	11.8	Refer.		[0.06 – 0.58]
Large	27	5.8	1.18		[0.05 – 0.65]

The occurrence of phantom cow syndrome (PCS) in animals that have particular reproductive disease such as dystocia, RFM, abortion, uterine metritis, pyometra, and lameness was progressive and categorized in (Table 3).

Table 3: Prevalence of PCS by reproductive disorder of dairy herds (n=720)

Disease	No. of farms	No. animals	Prevalence (%)
Abortion	6	9	10.5
Dystocia	18	23	26.7
Lameness and mastitis	21	28	32.5

Metritis/pyometra	11	12	14
RFM	17	53	61.6

4.2. Response of hormonal treatment of phantom cow syndrome

Respective to ovarian and uterine status revealed by ultrasonography examination, the overall efficacy of hormonal treatment of phantom cow was specifically high and potentially effective and most of phatom cows were became pregnant and returned to normal endocrine cycle. Table 4 show the ultrasonography revealed reproductive organs (uterus and ovary) disorder of phantom cows and appropriate hormonal treatments that induce the possible reproductive and endocrine cycle.

Table 4: Frequency of reproductive problem and corresponding hormonal treatments

Treatment group	Ovarian status	Uterine status	Hormonal Treatment protocols
G-I= 11 animals	Anovulatory	Normal	Ovsynch
G-II=11 animals	Inactive	Flaccid	Ovsynch + CIDR priming
G-III= 16 animals	Luteal cyst	Pyometra and metritis	PGF2α + uterine Lavage

The details response of each treatment protocols was indicated by the proportion of animal's pregnancy rate and returned to estrus within 22 days of post AI. Figure 10 show uterine (lavage) treatment by antibiotic (infusion of 20% Oxytetracycline), saline, and povidone iodine, guided by uterine douche and Foley catheter (Figure 9 and 10). Out of seven animals treated by uterine lavage, almost all animals were restored reproductive cycle (six animals become pregnant and one animal returned to second estrus cycle). The efficacy of PGF2alpha treatment together with uterine lavage was very high and 87% of treated animals were responded.

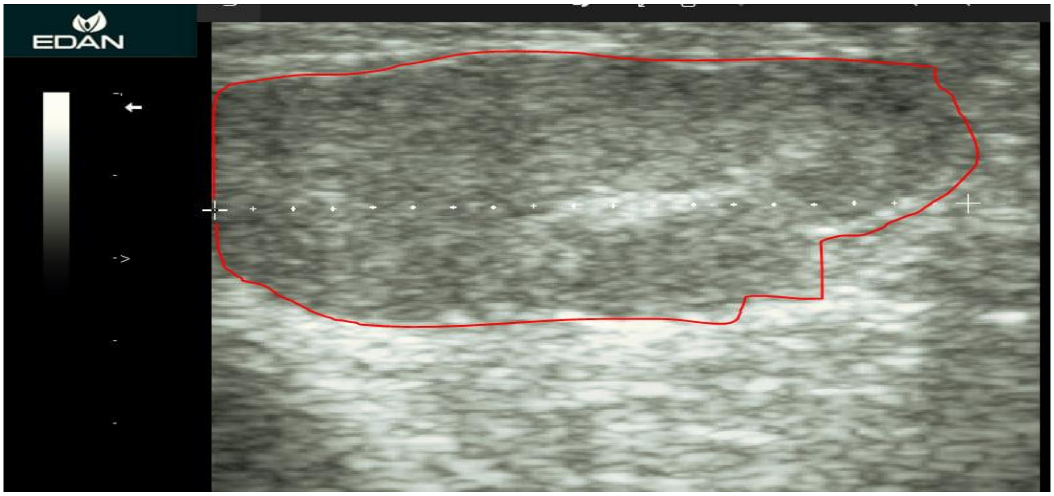


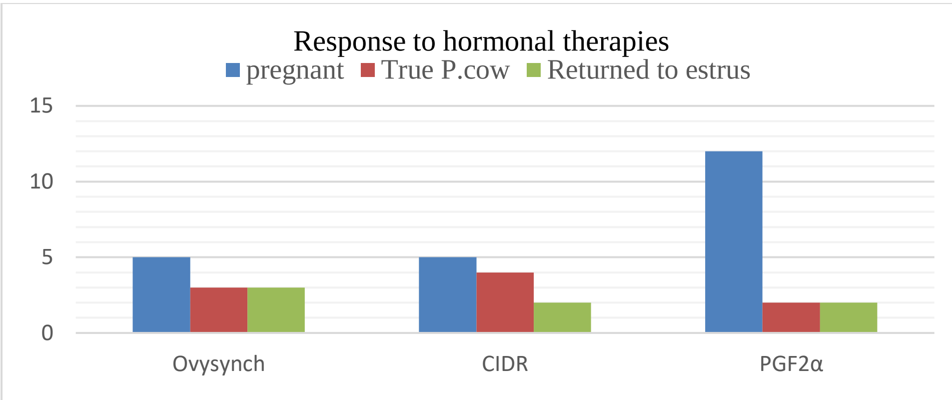
Figure 9: Ultrasonogram image of pyometra and metritis (filled uterine body red circle)

Figure 10: Uterine lavage of cow pyometra and metritis by uterine douche

The treatment of phantom cows by Ovsynch and Ovsynch priming CIDR protocols have revealed that both protocols have had the same efficacy to treat phantom cows and almost seventy seven percent of treated cows had restored their production progress and breeding (pregnant and returned to estrus).

4.3. Pregnancy rate and identification of true phantom animals

Figure 11: shows the particulars of the pregnancy rate of inseminated dairy cows by clinical examination (P4 Golden Test) of inseminated animals starting at 22 days post-treatment and finally confirmed by ultrasound examination later on days 36 of post AI.



Notes: True P.cow= true phantom cow

Figure 11: Efficacy of different hormonal treatments

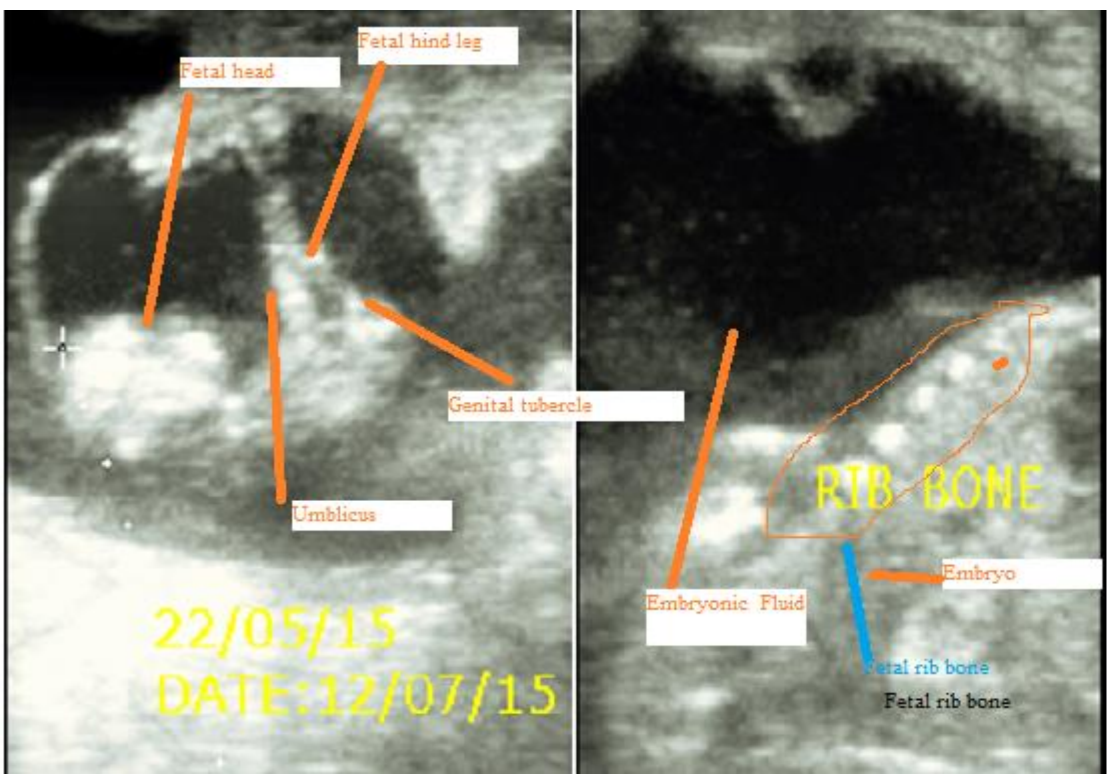


Figure 12: Ultrasonographic image of 50 and 52-days old embryo of treated animals.

Accordingly, there were 18.4% of cows classified as Phantom cows, while 57.9% were pregnant to the first serve and 23.6% returned for a second service within 22 days of the first service (Figure 13). There were short retuned on 17 days and long returned on 25-27 days of post AI to estrus after treatments.

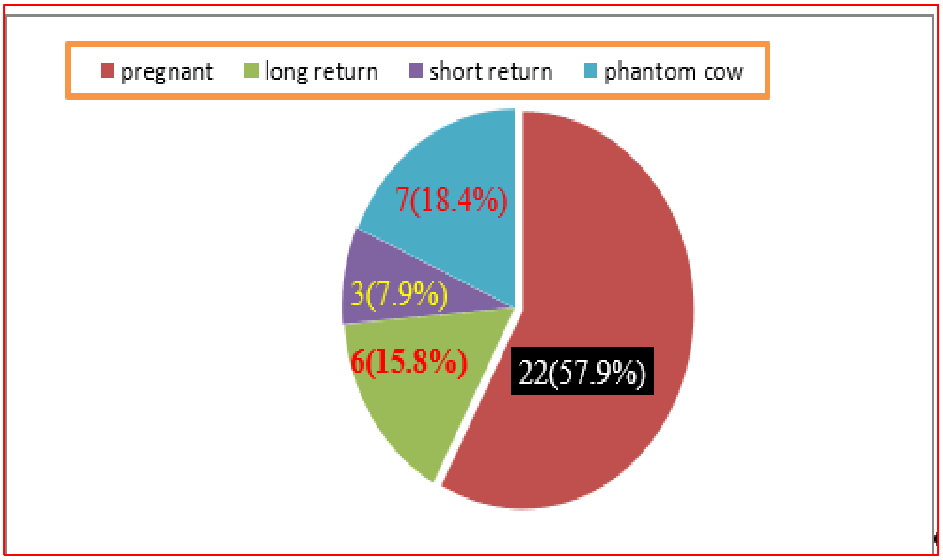


Figure 13: Diagram of proportion and percentage of hormonal treatment response

5. DISCUSSION

There was limited information about phantom cow syndrome worldwide and particularly in Ethiopia except few reports from Australia and New Zealand. Although previous information related to phantom cow management the results of the current study indicated that hormonal treatments intervention in phantom cow control confirmed that treating phantom cows with ultrasound-based appropriate hormonal treatment is appropriate and effective. It is economically feasible on dairy farms concerning age, BCS, parity, interval from calving to service, anestrous, herd size, and intervention according to the history of the animals before time.

The effort made during the current study was to determine the prevalence of phantom cow syndrome, identify the major risk factors inducing phantom cow syndrome, and evaluate the response of ultrasound-based hormonal treatment. It is a serious production and reproduction problem of dairy farms that causes huge economic loss for the dairy producer and creates a serious reproductive management challenge for the newly established dairy small farms. Phantom cows are cows under the state of non-cycling non-pregnant condition that affect reproductive performance of the dairy herd particularly in countries like Ethiopia where there is a lower trend of pregnancy checkups post insemination.

The overall prevalence of Phantom Cow Syndrome in the current study is higher than the previous finding in dairy farms in and around Bishoftu town (7.45%) by (Kassa, 2017). This study revealed that small holder dairy farms are more affected by PCS compared to the medium and large sized farms. Owing the type of management, the breeding system small holder farms seem to be more affected with PCS than medium and large dairy farms. The lack of accurate estrus detection in many small holder dairy systems in urban set has been repeatedly confirmed (Sign *et al* 2021). It is known that heat detection failure exacerbates the incidence of phantom cow syndrome in a herd since it means missed heat and phantom cow syndrome (Filho *et al*., 2010). Reports by other authors also confirm that up to 30% cows inseminated are not in estrus when bred (Leroy *et al*., 2008).

The higher prevalence in small dairy farms might be related to many animal related factors. Further, proper animal housing facilitates, optimal health; production and welfare of dairy animals are contributors of PCS (Phillips, *et al*., 2013).

The fact that the current finding is relatively higher than previous report by Kassa (2017) for the same place shows factors contributing to the occurrence of the problem has not changed much or could have actually increased in existence. Incidence of PCS elsewhere are still lower such as 9% and 50% reported from New Zealand dairy farms by Cuttance *et al*. (2015) and Chambers *et al*. (2020), respectively. Moreover, 22% and reported from Australia by Nation *et al*. (2001). In general, the disparity in these findings from different countries might be attributed to differences in the management system, use of biotechnology, epidemiological conditions, and variation of in feeding management, and the type of production system.

Clinical survey and record keeping data revealed, metritis/pyometra and abortion were highly prevalent in first calved animals following dystocia and RFM, and highly associated with the incidence of phantom cow syndrome. This might be due to calving management problem, nutritional deficiency (vitamin E, selenium, and carotene), immune suppression, and over-conditioned cows during mating/AI and parturition. Cows with RFM and dystocia had a significantly higher incidence of metritis/ pyometra and lameness (Nation *et al*., 2001; Cuttance and Mason, 2015; Opsomer, 2015). The incidence of lameness is higher in cows with the history of dystocia cases and poor housing. Twin birth and abortion had also negative impact on the reproductive performance and fertility efficiency dairy cows.

The significant age influence in the prevalence of PCS where young animals were more affected compared to the relatively older one has been previously reported. Factors such as age at puberty and sexual development are significantly impacted by energy deficit, conditions that are common in small farms, in young animals due to decreased endocrine activity (Phillips, *et al*., 2013). The incidence in young animals (<3 years old) was two-fold as higher compared to adult and old animals. This is in contrast to the study conducted by Kassa, (2017) where animals with higher parity are more affected than younger animals. Nutritional deficiencies, particularly related with energy and mineral requirements for postpartum cows.

Previous studies by Nation and his colleague (2001), showed 18% and 16% in two- and three-year-old animals, respectively, in the Maffra field experiment, Australia. Heifers were known to be most at risk of being anestrous, and there was a strong association between anestrous and Phantom cow syndrome in heifers and primiparous cows as compared to adult or older cows. The longer interval from calving to service strongly associated with PCS occurs as a result of management factors such as lack of detection of silent estrus and absence of teaser bull capable of accurately detecting estrus (Kadhim *et al*., 2020), nutritional (energy and protein) factors, endocrine hormone insufficiency, and congenital problems (ovarian hypoplasia) (Archbold *et al*., 2012) at extend the CCI and submission date.

The analysis of various factors showed the presence of a positive association between anestrous and incidence of PCS. This implied that the higher the number of anestrous cows the higher the incidence of PCS in a farm. Similarly, Nation *et al.* (2001) found that primiparous Holstein cows that were more likely to have anestrous in New Zealand had the greatest incidence of PCS. PCS was also more associated with relatively higher daily milk yield. Cows that were producing milk >12L per day at early and mid-lactation period were six time more affected by PCS.

Overall, more than half of the cows were confirmed pregnant at first service, 23.7% returned to service, and 18.5% were diagnosed as non-return, non-pregnant cows (phantom cows). The majority of cases of phantom cows were explained as having either anovulatory condition (not returning for service) due probably to higher incidence of luteal cysts and pyometra (reverting to an anestrous state after the disease). As evidenced by higher proportion of pregnant animals confirmed by P4 Golden test and ultrasonography after treatment shows PCS is treatable condition. The lack of cycling in phantom cows might be the direct result of hormonal disturbance caused by the various risk factors. It can also be noted that early pregnancy diagnosis as a reproductive management tool can make a significant difference in restoring non-cycling animals or higher submission of failed animals that were inseminated in the preceding cycle. The use of a more accurate pregnancy diagnosis tool such as ultrasound system or easier rapid test such as P4 Gold test in detecting pregnancy or non-pregnancy is instrumental in reducing the incidence of PCS.

Treatment with PGF2 alpha was relatively better when coupled with uterine lavage as evidenced from the higher pregnancy rate. A few animals still returned to estrus indicating cyclicity has been restored while the condition did not improve in those that did not retun to cycle or became pregnant (the true phantom cows). It probably shows that not all cases of Phantom cow syndrome are mediated by hormonal disturbances. The factor behind the occurrence of some kinds of PCS needs to be further studied as this study confirms the presence of different kinds or classes of PCS based on the actual predisposing factor.

Results from this experiment maintain the conclusion that dairy cows need to be treated with appropriate hormones to achieve an approximately 50% conception rate, return to service, and decrease the calving interval of phantom cows. Although this is the first study considering the efficiency of hormonal therapy in phantom cow treatment intervention, and our results still confirms treating phantom cows with appropriate hormonal treatment is feasible.

6. CONCLUSSION RECOMMENDATION

Phantom cow syndrome is a serious production and reproduction risk for dairy farms that causes huge economic loss for the dairy producer and creates a serious reproductive management challenge for the newly established dairy farms (small). As evidenced in this study, poor reproductive efficiency, disrupted calving pattern, fewer replacement, loss of production, and lost income for farms due to animal age related, parity, herd size, uterine infectious, ovarian problem and management related risk factors would become a major challenge for the small holder as this is a newly emerging dairy production system designed in improving milk productivity in Ethiopia. Even with lesser prevalence, it can still pose huge economic loss by hindering dairy expansion because of the reduced calf crop and extended calving interval. The risk factors identified in this study are worth considering when optimizing the production system. Many of the risk factors identified in this study are more common in the small holder dairy farms thus need to be considered during optimizing the production system. Age at puberty, management of postpartum cows, and adjustment of feeding management particularly as the herd size increases are important aspects of mitigation plans. The use of a more accurate method of early pregnancy detection can also contribute to the reduction in the incidence of PCS as there are increased chances of submission of failed animals. Proper diagnosis of phantom cows and their early treatment using hormones can potentially reduce the impact of PCS

The following recommendations are forwarded:

- Proper estrus detection and early pregnancy diagnosis must be practiced in all farms to reduce the incidence of PCS
- The practice reproductive herd health management program where most of the risk factors identified in this study can be dealt with can dramatically change the impact of PCS on productivity
- Installment of hormonal therapy for phantom cows can also be an option to further reduction of the influence of PCS by restoring non-return cows to cyclicity
- Further, detailed investigations of PCS and experimentation on treatment are required to classify the syndrome based on the primary causes and to design an appropriate therapeutic and control method.

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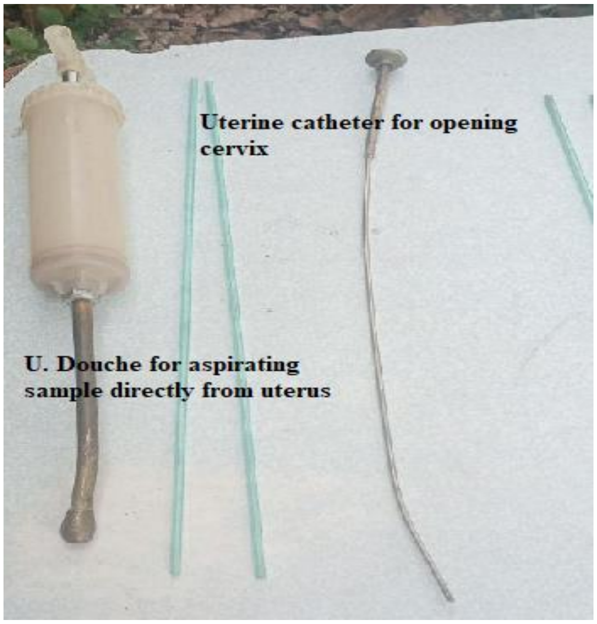
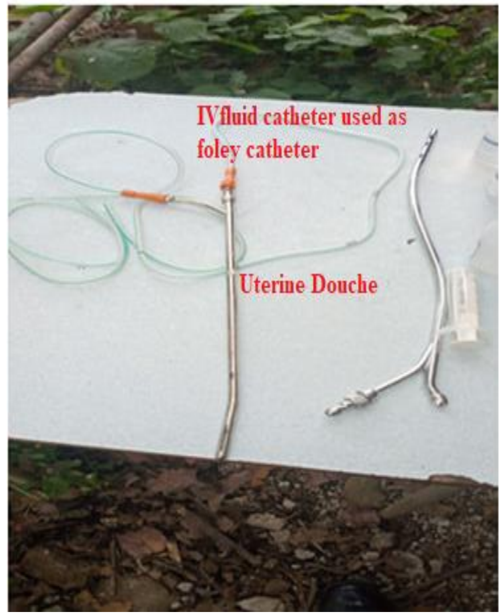
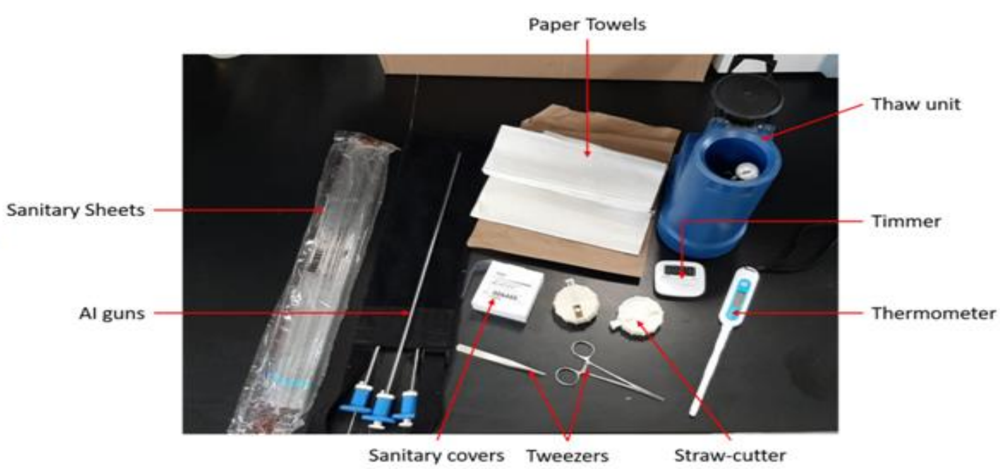
8. APPENDEXES

Appendix 1: Photo captured during the treatment of phantom cows at different farms by using different techniques.

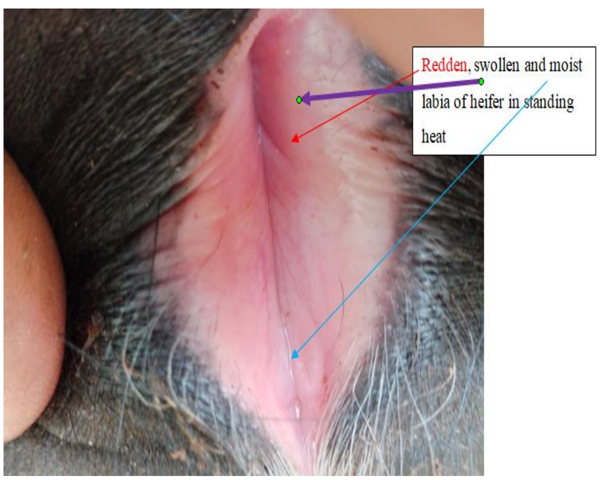




Appendix 2: AI Kit and principles, ultrasound examination and semen thawing technique

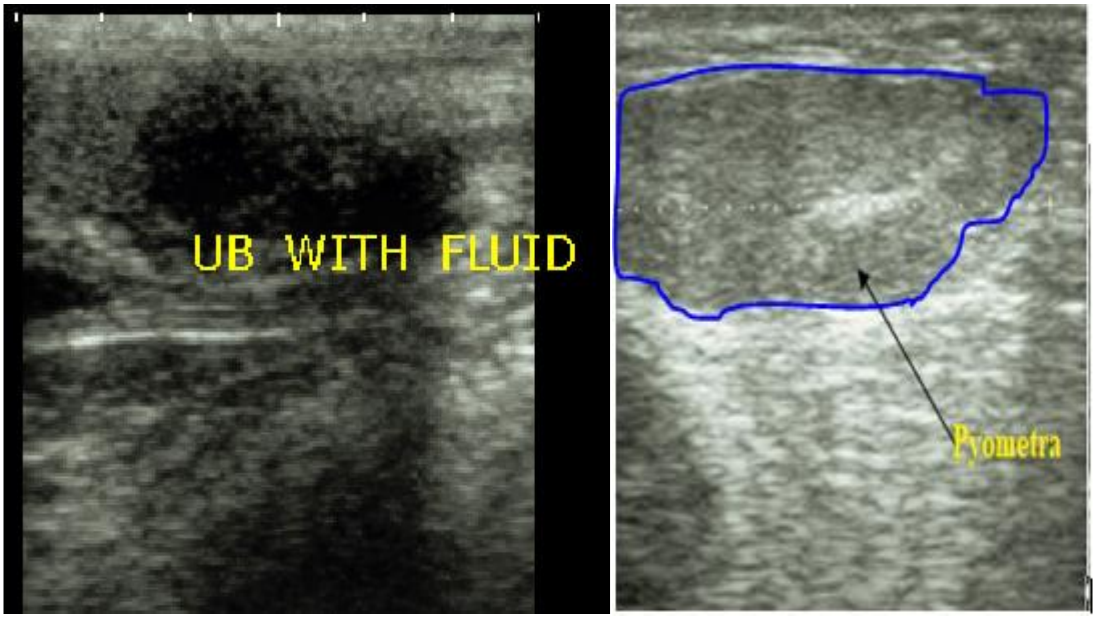


Appendix 3: Major behavioral estrus sign of PGF2alpha treated animals during observation by follow up.

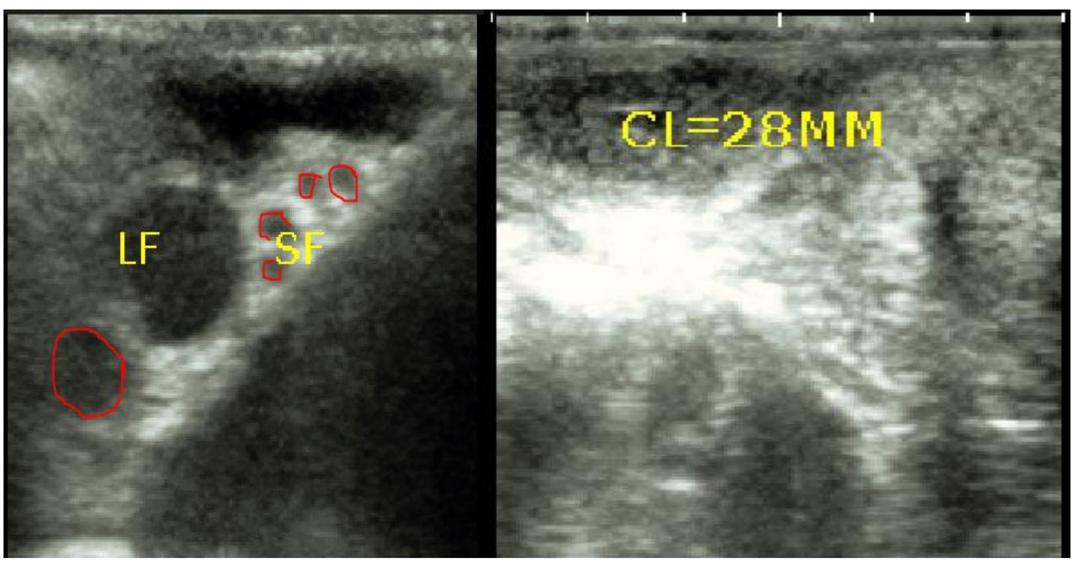


Appendix 4: Image/ photo captured during the treatment of phantom cows at different farms.

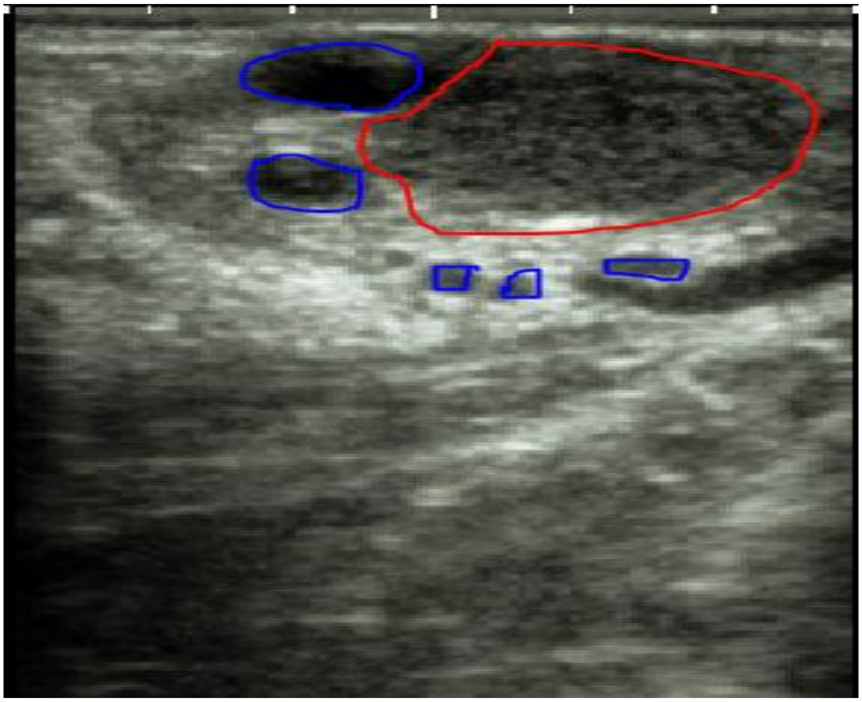
Ultrasonography results of uterine metritis/pyometra lavage by saline water and antibiotic and reddish- brownish uterine discharge through Foley catheter



A. Abnormal condition of uterine body

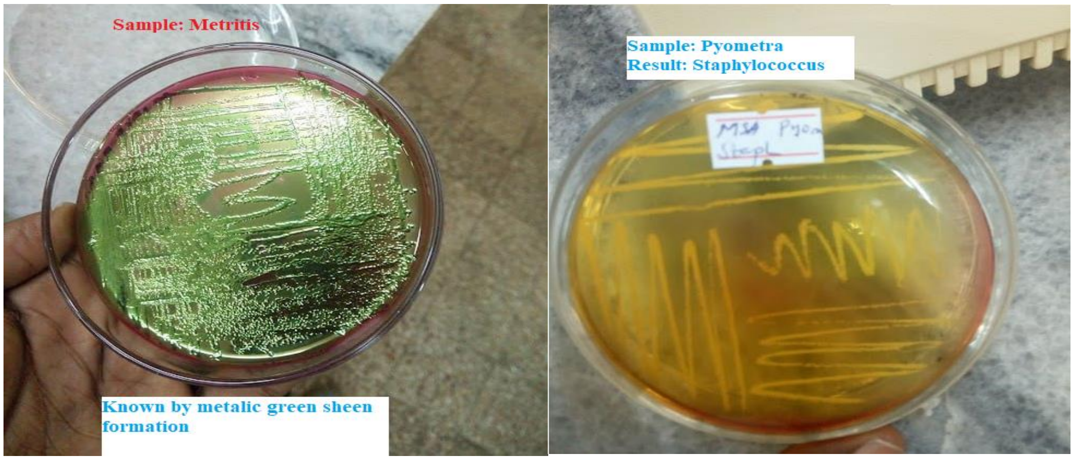


A. Some ovarian abnormality (anovulatory follicles and luteal cyst from left to right respectively) revealed by ultrasound. Red circles indicate medium and small follicles and LF represent large and an ovulated follicle.



B. Images of persistent CL surrounded by different size of follicles (blue circles) of ultrasound scan

Image of uterine lavage and laboratory result of uterine content sample



Isolation of *E. coli* on EMB (a) and MacConkey (b) media; isolation of *Staphylococcus* bacteria on MSA media. Selective media

Ultrasonophic image of pregnant animals

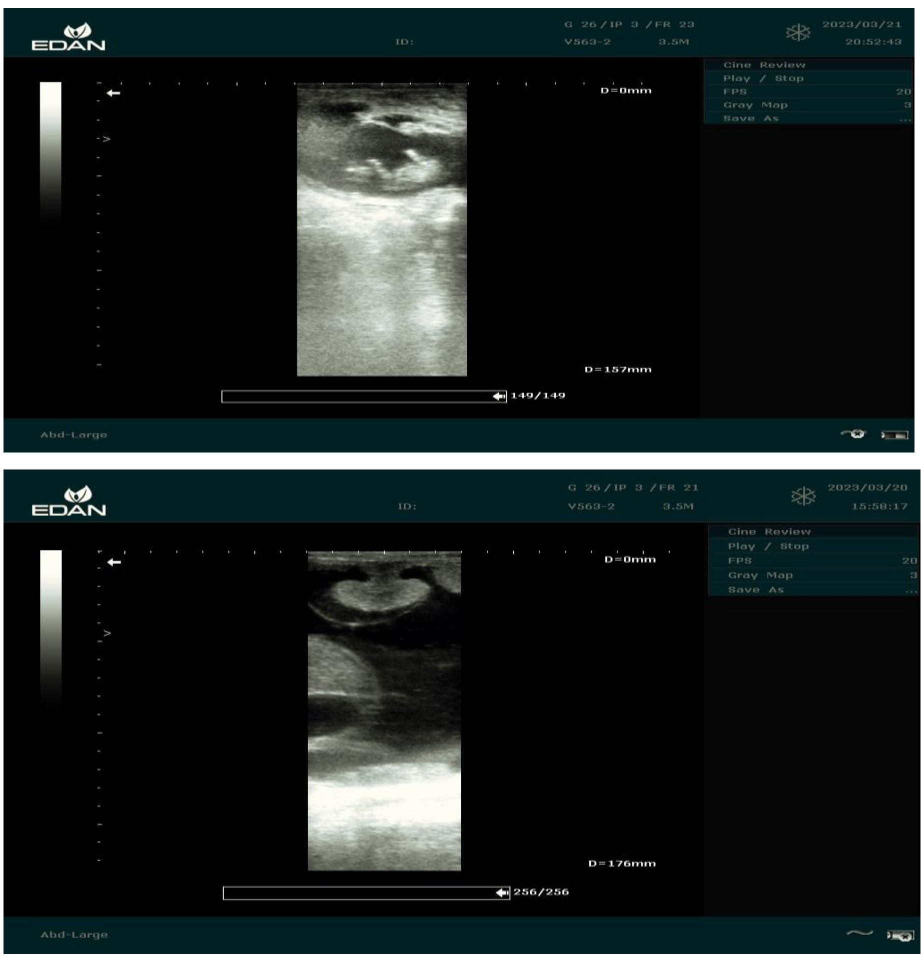


Image of 56 days old embryo and placenta of 35-day old pregnant animals

Appendix 5: Ethical clearance approval certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/24/04/15/2023

Name and affiliation of applicant: **Onneta Regasa Gelan (DVM, MSc student)**
Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project*: Prevalence, major risk factors and response to hormonal therapy of phantom cow syndrome in urban and peri-urban dairy farms in Bishoftu town.*

Date of application:	**November, 2022**
Nature of the project:	**Field investigation**
Target animal species:	**Cattle**
Number of animals involved:	**720**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/15/023, 15/02/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Onneta Regasa Gelan.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

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