Potency of Phitofert in Improvement of Reproductive Efficacy in Sulpiride-Treated Rats

Nahla J. M. S. Al-Shahery

Abstract

The current study was designed to investigate the persisting of hyperprolactinaemic (Hprl) symptoms because the impact longstanding of sulpiride injection (the 2nd model of Hprl), to prove these symptoms when they compared with these in 1st model of Hprl. Also we investigated whether Hprl had ability to induce altering in the activity of ovarian hydroxy steroid dehydrogenase (HSD) enzymes and oxidative status in the ovarian tissue. The goal of this study was to evaluate the potentiality of phitofert product in adjustment of Hprl status for each 2nd model of Hprl. Therefore, female rats were divided as follows: G1: female rats were injected with normal saline (0.9% NaCl) for 28 successive days (control). G2: Female rats were injected with 40 mg/kg b.w. of sulpiride for 28 successive days (as a first model). G3: Female rats were injected with normal saline (0.9% NaCl) for 28 successive days, and they were orally administrated with d.w. for another 28 successive days (controls). G4: Female rats were injected with 40 mg/kg b.w. of sulpiride for 28 successive days, and they were orally administrated for another 28 successive days with d.w. (as a second model). G5: Female rats were injected with 40 mg/kg b.w. of sulpiride for 28 successive days, and they were orally administrated with 7 mg/kg b.w. of phitofert for another 28 successive days. The hyperprolactinaemia signs primarily a significant (P≤ 0.05) elevation in the serum prolactin (Prl) and progesterone (P) levels were found, while the serum FSH, LH and estrogen levels were found to be significantly (P≤ 0.05, P≤ 0.001) decreased in G2 and G4 than controls (G1 and G3). The hyperprolactinaemic rats (G2 and G4) also, showed a significant (P≤ 0.05) decreased in the activity of an ovarian 20α-HSD enzyme, compared to G1 and G3. The oxidative stress (OS) as evidenced by significantly (P≤ 0.05) increased in the activity of lipid peroxidation (MDA) and decreased in the ovarian activity of GSH and CAT levels were found in these groups. Also the estrus cycles and ovulation process were ceased in hyperprolactinemic groups (G2 and G4). Whereas the results of G5 showed that, the phitofert had a positive role in the reverse of all above assessed parameters in sulpiride-induced hyperprolactinemia groups. The phitofert treatment resulted regular estrus cycle and the restoration of ovulation, ditto the ovulated oocytes were able to fertilize in vitro. These fertilized oocytes of G5 were succeed in the development in vitro to blastocyst stages. So the transferred blastocysts into recipient females were able to implantation compared to those in control (G3). Thus, our findings proved the ability of phitofert product in therapy of HPRL.

Introduction

Prolactin hormone is created and secreted from the major source i.e. lactotrophic cells of anterior pituitary [1]. This hormone possesses various biological functions such electrolyte balance, development, behaviour, immune regulation, metabolism and reproduction [2]. Also, its over secretion that nameable hyperprolactinemia can be occurred via either by physiological events [3, 4], or pathological and pharmaceutical conditions [5, 6].So hyperprolactinemia can be induced experimentally using some substance i.e. metoclopramide [7] and antipsychotic drugs such chloropromzine [8] and sulpiride [9]. Hyperprolactinaemia was diagnosed as a common endocrine disturbance in nearly third of infertility female [10]. Whereby it led to disrupt of reproductive function in male [11,12] and female [13].

So, excess prolactin levels resulted several symptoms such, lowering of gonadotropin levels, gonadal dysfunction [14] galactorrhoea, amenorrhea, anovulation [10,15], insufficiency lutal phase and dysfunction of follicular phase that led to infertility [16,17].
In addition, it caused failure mating and decrease pregnancy rate in female rats with hyperprolactinemia [18]. It had been reported that, many drugs was used as dopamine agonist to lower prolactin levels and restoring of reproductive efficiency like, bromocriptine [19], pergolide [20] cabergoline [11] and quinagolide [21] but they caused various morbidity symptoms as neurological effect [22].

The phitofert Donna product (180 cps) for woman was manufactured in UE for promopharm s.p.a. and it used as dietary supplements. This product was useful as assistant to ameliorate fertility and to have adaptogenic and sexual tonic action in female and male because of its components particularly macca and folic acid. The gradients of phitofert include 3000 mg of maca dry extract (Lepidium meyenni w., tubero), 180 µg of folic acid, microcrystalline cellulose, colloidal silica and magnesium stearate (Info a promopharma.It- w.w.w.promopharma.it). As it was known that maca (Lepidium meyenni) plant belongs to (Brassicaceae) and growing in South America. For many centuries, it had been used as food, traditional medicine as remedy several diseases [23, 25] and it was used to cure infertility in both sex via increased fertility in several species [26].

For the second gradient i.e folic acid, it was reported that, folic acid was a form of vitamin B-9, and to have many vital roles in the synthesis and repair of DNA and RNA. So it was aiding rapid cell division and growth [27, 28].

Therefore, this study aimed for the first time to investigate the possibility of phitofert product for woman to cure the infertility symptoms of the sulpiride-induced hyperprolactinaemic rats as alternative remedied about prementioned medicines to avoid its passive impacts.

Materials and Methods

Fifty adult mature female and 10 male rats of Sprague-Dawely (150-200 g) were housed under temperature 25-25 °C controlled lighting (14L:10D). Pellets and water were supplied ad libitum.

Induction of Hyperprolactinemia

Sulpiride (Delagrange, France) was used to induce two models of hyperprolactinaemia (Hprl) by injection of 40 mg/kg b.w. intraperitoneal. This dose used according to Mostafapour et al. [18].

Experimental Layout

Normal female rats were divided into 5 groups as follows (n=8):
G1: animals were injected with 0.1 ml of normal saline (NaCl% 0.9) for 28 successive days.
G2: In the first model of animals were injected with 40 mg/kg b.w. of sulpiride for 28 successive days.
G3: female rats were injected with 0.1 ml of normal saline (0.9% NaCl) for 28 successive days, and they were orally administrated with d.w. for another 28 successive days (Control).
G4: In the second model of animals were injected with 40 mg/kg b.w. of sulpiride for successive 28 days and they were oral administrated with 0.1 ml of d.w. for another 28 successive days.
G5: animals were injected with 40 mg/kg b.w. of sulpiride for 28 successive days and they were orally administrated with 7 mg/kg b.w. of phitofert for another 28 successive days.

At the end of all experiments, blood collected by heart puncture and offed to clot for 2h. Serum was obtained post centrifugation for 10 minutes at 3000 rpm and conserved at -20 C° for hormonal analysis.

Hormonal Assays

Serum levels of Prl, FHS, LH, E and P were examined by using automated instrument TOSOH. And the kits of these hormones from TOSOH Bioscience Company, so the immunoenzymometric assay (IEA) were used.

Assessment of Ovarian Enzymes Activities

Twenty ovaries of all five groups were excised to estimate of 20α-hydroxysteroid dehydrogenase (20α-HSD and 3β-hydroxysteroid dehydrogenase 4 (3β-HSD) activities, according to the method of. Also, other 30 ovaries of a above groups were homogenized with KH2PO4 buffer (100 µM) with EDTA (1 μM, pH 7.5) and there, the homogenate of ovaries was centrifuged at...
13000 g for 20 at 4 °C, the supernatant was used to determinate of oxidant enzymes lipid peroxidation (MDA) activity as well as the antioxidant agents such GSH and the best of antioxidants enzyme i.e., catalase (CAT) activity according to the methods of [29, 31].

**Preparation of Caudal Sperm and Vasectomized Rats**

Woofy epididymal mass of sperms of 5 adult male rats was obtained by minced gently for their cauda epididymidis. It was entered into 400 ml of mKRB that had been covered with paraffin oil and counterbalanced at 37 °C in humid atmosphere of 5% CO₂ in air. Approximately 5 min post preparation 50 μl of sperm suspension was converted into 400 μl at the fertilization medium (sperm concentration of 0.5–1×10⁶ sperm/ml).

This diluted sperm suspension was preincubated for 5-6 h at 37°C and 5% CO₂ in air [32]. In addition, the vasectomized rats (n=5) were done according to [33]. The vasectomized rats (n=5) were done according to [33].

**Evaluation of Ovulation Rate and IVF**

For appraise of the ovulation rate, female rats of all groups were sacrificed between 7-8 h at the estrus day. Oviducts were separated from ovaries to get of cumulus oocytes complexes (COCs).

The mature oocytes (Figure-1) were stripped from COCs by piptting with 0.1% hyaluronidase in mRIECM and entered into the diluted sperm suspension which covered with paraffin oil, then cultured for 10 h at 5% CO₂ incubator at 37°C.According to Oh et al. [32]. Ditto, the normality and number of oocyte were evaluated.

**Evaluated of in Vitro Embryonic Development and Embryo Transfer**

The denuded oocytes that have two pronculi were considered to be fertilized by using inverted phase-contrast microscope (Olympus).

The fertilized oocytes were washed 3 times by MRIEC medium. Oocytes (no= 10) were transferred into 50 μl of MRIEC culture medium at 37 C° and 5% CO₂ in air for pursuing fertilised oocytes development in vitro. They were examined post-different interval time i.e, 42, 72, 96 and 120 h of culture to determine the rate at which embryo had developed to 4-cell, 8-cell, morula and blastocysts.

**Embryo Transfer**

To assess in vivo development and then implant of IVF resulted embryos of G3 and G5, 10 pseudo pregnant female rats were prepared by mated them with vasectomized males to induce pseudo pregnancy as recipient females. Al-cm incisions in the left and right flank were made and delivered the uteri. Ten embryos that had develop to blastocysts post 120 h of culture were transferred with fine glass micropipette into each uterus (5/horn).

The uterine horns were replaced in the peritoneal cavity and the muscle and skin were sutured. All recipient female rats were
sacrificed at mid gestation: 14 days post embryo transfer [33]. The uteri were excised and examined the implantation sites.

Statistical Analysis

Our data were expressed as mean± S.E. results were compared by analysis variance (ANOVA) and t-test with using SPSS (v11.5) (IBM SPSS static software. The different between data with P value under 0.05 was considered statistical.

Results and Discussion

The influence of Phitofer on Hormonal Profile and Ovarian Enzymes Activity for Hyperprolactinaemic Rats

Table-1 showed that the use of sulpiride to induce, Hprl models (G2 and G4) led to perturbation in the serum Prl, gonadotropins (Gns) and steroid hormones levels, in addition induced changes in ovarian SDH enzymes activity and appearance of ovarian oxidative stress.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Prl ng/ml</th>
<th>FSH mIU/ml</th>
<th>LH mIU/ml</th>
<th>P ng/ml</th>
<th>E2 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>22.10 ± 1.41 a</td>
<td>6.21 ± 0.12 a</td>
<td>7.03 ± 0.05 a</td>
<td>11.10 ± 0.14 a</td>
<td>4.00 ± 0.25 a</td>
</tr>
<tr>
<td>G2</td>
<td>60.03 ± 0.31 b</td>
<td>2.90 ± 0.19 b</td>
<td>3.20 ± 0.32 b</td>
<td>22.00 ± 0.30 b</td>
<td>0.10 ± 0.03 c</td>
</tr>
<tr>
<td>G3</td>
<td>20.00 ± 0.11 a</td>
<td>5.12 ± 1.10 a</td>
<td>7.00 ± 0.01 a</td>
<td>10.21 ± 1.2 a</td>
<td>4.12 ± 0.23 a</td>
</tr>
<tr>
<td>G4</td>
<td>59.01 ± 0.98 b</td>
<td>3.30 ± 0.34 b</td>
<td>4.10 ± 0.28 b</td>
<td>25.02 ± 0.17 b</td>
<td>0.11 ± 0.05 c</td>
</tr>
<tr>
<td>G5</td>
<td>19.00 ± 0.54 a</td>
<td>5.03 ± 0.40 a</td>
<td>6.55 ± 0.13 a</td>
<td>9.00 ± 0.07 a</td>
<td>3.10 ± 0.08 a</td>
</tr>
</tbody>
</table>

b means significant at P≤ 0.05.
c means significant at P≤ 0.001.

Table 2: Impact of phitofer in the activity of ovarian enzymes

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Activity of ovarian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20α-HSD mU/mg</td>
</tr>
<tr>
<td>G1</td>
<td>9.5 ± 1.20 a</td>
</tr>
<tr>
<td>G2</td>
<td>4.00 ± 0.10 b</td>
</tr>
<tr>
<td>G3</td>
<td>8.53 ± 0.11 a</td>
</tr>
<tr>
<td>G4</td>
<td>3.73 ± 0.63 b</td>
</tr>
<tr>
<td>G5</td>
<td>8.82 ± 0.08 a</td>
</tr>
</tbody>
</table>

Different letters mean significant difference at P≤ 0.05 between groups'.

In this study, the elevated P hormone could be linked with a significant (P≤ 0.05) increment in the activity of ovarian 20α-HSD enzyme (which involves in the progesterone catabolism) in hyperprolactinaemic rats (G2 and G4) compared to G1 and G3, while the Hprl did not change the activity of ovarian 3βHSD enzyme (which involves in the progesterone anabolism) (Table-2).

Additionally, the treatment with sulpiride (G2 and G4) caused a significant (P< 0.05, P< 0.001) reduction in the serum FSH, LH and oestrogen (E2) levels compared to G1 and G3.

So we believed that the excess of Prl production may had a negative influence either on the pituitary gonadotropic cells or by the reduction in hypothalamic GnRH as confirmed by Sonigo et al.[36].

Whereas, the decrement in the serum E2 levels in these Hprl groups may be attributed to the regression in the follicular phase, i.e the declining in the number of developing follicles, which was considered as a major source for E2 secretion.
Thus the similar results of G4 with those of G2 confirmed the persistence impact of sulpiride to induce Hprl even for 28 days post the end of its injunction. As shown in Table-1, the phitofert treatment for 28 days post the last of sulpiride injection (G5) showed significant (P≤ 0.05) reduction in both Prl and P while, it offered significantly (P≤ 0.05, P≤ 0.001) elevation in FHS, LH, E2 and the activity of ovarian 20α-HSD compared to G4.

The almightiness of phitofert to modulate of sulpiride-induced hyperprolactinaemia symptoms may be arrose via its gradients, particularly one of its most important which was maca plant. Previously, maca was considered to have lots of bioactive components with medicinal properties, such as glucosinolate, macaenes and maca [37], in addition to protein, amino acid, lipid, minerals, alkaloids and hexadecanamide [38].

Chung et al. [39] reported the alkaloid of maca to have influence on increasing libido and fertility in adult rats. Therefore we suggested that, the phitofert may decrease the affinity of sulpiride for dopamine receptors, or it affected hypothalamus-pituitary axis, and leading to enhancement the releasing of dopamine which resulting to the decrement Prl secretion and restoration its normal values.

This explanation is supported by the finding of Ai et al. [40] who demonstrated that the treatment with maca (main gradient of phitofert) for 6 weeks led to the significant elevation in dopamine levels in mice. Conversely, the reduction in Prl levels in phitofert-treated hyperprolactinaemic rats (G5) was responsible for the elevation of the activity of ovarian 20α-HDS enzyme and the resumption to its normal value compared to its value in G4.

Thus, in G5 the phitofert therapy appeared to be important in regression of hyperprolactinemia-induced like luteal phase which leading to a significant (P≤ 0.05) reduction in the P hormone levels compared to G4. Meanwhile the phitofert treatment (G5) produced a significant (P≤ 0.05, P≤ 0.001) increment in the levels of FSH, LH and E2 hormones and restoration them to their normalcy compared to G4. Even these values were similar to those in normal cycling group: control (G3).

In this study, the potency of phitofert to adjust FSH and LH levels may be as a reflection of Prl evert to its physiological levels. This finding was in accordance with the study by Uchiyama et al. [41] who reported that, maca-treated female rats exhibited a significant elevation in each FSH and LH levels at the proesturs stage, which led to enhancement of fertility.

For the significant (P≤ 0.001) elevation in E2 levels in G5 could be linked with the balance of gonadotropin hormones post phitofert treatment. These results confirmed many previous studies as, a study by Kumar and Farouk-Sait [42] who revealed that each of gonadotropins was shared in estradiol outputting via folliculogenesis. So with Saleeon et al. [43] who recorded the elevation in E2 levels in proestrus and estrus stages.

The Influence of Phitofert on Recylicity of Hyperprolactinaemic Rats

Hyperprolactinaemic rats (G2 and G4) demonstrated the blocking of ovulation process, and they had no apparition of oocytes via collecting operation compared to controls (G1 and G3) (Table-3).

As shown in Figure-2, this logicality result was agreed with the stopping of estrus cycle (predominant of hyperprolactinaemia like-luteal phase) and the absence of follicular phase (Arresting of follicular development) which resulting to an ovulation. This finding was consistence with the result of Sonigo et al. [36] who reported the ban ovulation on Prl-treated mice (as hyperprolactinaemia model) and it attributed to the significant decrease in pituitary FSH transcript and serum LH and FSH levels.

Also, it was accordance with Goldman et al. [44] who reported that the follicular development not only needs to FSH as a major requested, but it needs to LH hormone in the late follicular phase to complete of ovulation process.

We suggested the regression in the follicular phase and then an ovulation in hyperprolactinaemac rats: G2 and G4, yielding from the disturbance in the ovarian oxidant-antioxidant balance resulting to oxidative stress. In accordance with the study of Alpay et al.[45] who reported that OS was a product of ROS over production and low antioxidant levels in infertility female with
endometriosis. As shown in Table-2, Hprl induced a significantly (P≤ 0.05) elevation in formation of ovarian lipid peroxidation (MDA), so this result evidenced of excess production of reactive oxygen species (ROS). Ditto, Hprl caused a significant (P≤ 0.05) lowering in the activity of ovarian GSH and CAT, in G2 and G4 compared to G1 and G3. And this declining indicated to the decrement of its antioxidant ability which may be incapable of scavenging the generation of ROS. Our finding was in agreement with the result of Veena et al. [46] who reported the positive correlation between serum Prl and oxidative stress marker such MDA and ferric reducing antioxidant power in serum of infertility women. In addition the high formation of ovarian lipid peroxidation (MDA) was considered as one of marker of oxidative tissue defect, become it could be able to induce free radical defect to the components of cellular membrane resulting to necrosis or apoptosis in the ovarian follicular cell.

Table 3: Impact of phitofert on oocytes outcome and in vitro fertilized oocytes

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total no. Of oocytes (ovulation rate mean ± S.E)</th>
<th>no. of normal oocytes</th>
<th>no. of abnormal oocytes</th>
<th>no. of inseminated oocytes</th>
<th>no. of fertilized oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>83 a (10.37 ± 1.21)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G2</td>
<td>0.00 b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G3</td>
<td>81 a 10.22±1.88</td>
<td>79 a</td>
<td>3 a</td>
<td>79 a</td>
<td>69 a (87.34)</td>
</tr>
<tr>
<td>G4</td>
<td>0.00 b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G5</td>
<td>81 a (10.12 ± 1.23)</td>
<td>78 a</td>
<td>3 a</td>
<td>78 a</td>
<td>66 a (84.61)</td>
</tr>
</tbody>
</table>

Different letters mean significant difference at P≤ 0.05 between groups'. Similar letters mean non-significant at (P≥ 0.05) between groups'.

According to Chaube et al. [47] who reported that, the increment ROS (H2O2) led to increase, in the caspase-9 and caspase-3 activities which trigger DNA fragmentation and thereby incidence oocyte apoptosis. While, the remedying with phitofert (G5) caused to supress the blocking of ovulation process and the restoration of the regular estrus cycles (Figure-2). Additionally, the total number and normally of recovered oocytes were symmetry to those in normal cycling rats (G3) (Table-3).

These results were to be as reflection for phitofert capability to decline in the Prl levels and restoration of gonadotropin hormones and E2 to normality status, then the restore of follicular phase that equips normal mature follicles. The adhering to this explanation evidenced by Insler et al. [48] who reported that, FSH was necessary for ovarian folliculogenesis stimulation, so the catalysing of LH could be crucial for optimal follicle and oocyte development. Also, it was known that, E2 hormone possessed a role in controlling the size of oocyte and primordial follicle pool in mice [49].

Moreover it was clear that, the ability of phitofert to reduce Prl levels led to reversing of imbalance of oxidant-antioxidant status in ovarian tissue.
Wherein we obtained a significant ($P \leq 0.05$) reduction in the activity of MDA levels in addition to a significant ($P \leq 0.05$) increase in the activity of ovarian GSH and CAT enzyme in G5 compared to G4 (Table-2).

Thus, we could linked between the high significant in the ovarian GSH and CAT activity and the increment in FSH and E2 levels in prevention the regression of follicular cells and then occurrence normal development and restoration of follicular phase which leading to ovulation. This finding was in agreement with Behl and Pandey [50] who reported the concomitant increase in catalase and E2 in response to FHS led to suggest that catalase had a role in follicular selection and prevention of apoptosis.

The possible mechanism of phitofert ability on attenuation of oxidative stress and improvement of antioxidant status in hyperprolactinaemic rats (G5) may be due to its gradients "maca".

Wherein Ai et al. [40] reported that, the treatment with maca extracts (250 and 500 mg/Kg) for six weeks caused the reduction in ROS in brain tissue of mice. Also, the result of Yang et al. [51] who reported that the administration of the most important active component of maca: macamides (12 and 40mg/Kg) for 3 weeks resulted significant decrease in the MDA activity in brain, muscle and a liver of mice. When tested the potency of normal recovered oocytes from phitofert-treated hyperprolactinaemic rats (G5) to fertilize and development in vitro we found they recorded a similar succeed to fertilize in vitro as those noted in G3.

And the capability of fertilized oocytes to develop in vitro to different stages of development i.e., 4-cell, 8-cell, morula and blastocyst stages were similar to those observed in G3 at the same time (Table-4, Figures-3, 4, 5, 6, 7, 8)

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total no. of cultured fertilized oocytes</th>
<th>No. of developing embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4-cell (42 h)</td>
</tr>
<tr>
<td>G3</td>
<td>69 a</td>
<td>65 a (94.20)</td>
</tr>
<tr>
<td>G5</td>
<td>66 a</td>
<td>62 a (93.94)</td>
</tr>
</tbody>
</table>

Similar letters mean non-significant at ($P > 0.05$) between groups.

Table 4: Development of fertilized oocytes in vitro for phitofert-treated hyperprolactinaemic rats

Figure 3: Fertilized oocyte (2 pronuclei) for G5, (40x)

Figure 4: Morula stage of G5, (40x)
In this study the ovulated high quality oocytes may result from the restoration of follicular development by modulation of OS and the influence of normalcy of reproductive
hormone that means the availability of follicular fluid which shored the maturation of oocytes preovulation.

Ditto, previously, it was found that, the biochemical traits of periphery follicular fluid with oocyte may have a critical influence in limiting oocyte quality and its achievement of IVF and embryos development [52].

**Implantation of Embryos Resulted by IVF of Phitofert-treated Hyperprolactinaemic Rats**

About the capability of blastocysts which produced by IVF of phitofert-treated hyperprolactinaemic rats (G5) to implant post transferring was similar to those observed in control G3.

As all recipient female rats became pregnant, and no significant (P> 0.05) difference in the implantation rates between them (G5 and G3) was noticed (Table-5, Figures-8, 9). This result confirmed that, the succeeding of IVF which led to incidence of pregnancy post blastocysts transfer into recipient females. And this finding was resulted of the good quality of oocytes.

While, Hourvitz et al.[53] Mentioned that, the poor quality oocytes maybe the causation of infertility. So we suggested that, the ability of phitofer to obtain good quality oocytes which able to implant may be attributed to its gradients “maca”. According to the study of Ruiz-Luna et al.[54] who reported the role of maca in improvement the number of offspring in mice. And maca was able to amelioration quality of mice embryos [26].

It was also suggested that the ability of phitofert to cure the hyperprolactinaemic signs in this study may be ascribed to the second gradient of phitofert i.e, folic acid, which based on the study of Nordqvist [28] who mentioned that, the role of folic acid was well-known in the cell division and growth. Therefore, the folic acid gradient maybe subsidizes the growth of follicular cells via follicular stage.

So we believed that, there was a synergistic action for both the components of phitofert i.e, maca and folic acid enabled to healing the hyperprolactinaemic symptoms.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total no. of transferred embryos</th>
<th>Total no. of implantation sites</th>
<th>The percentage of implanted embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3</td>
<td>50 a</td>
<td>40 a</td>
<td>80 a</td>
</tr>
<tr>
<td>G5</td>
<td>50 a</td>
<td>37 a</td>
<td>74 a</td>
</tr>
</tbody>
</table>

Similar letters mean non-significant at (P> 0.05) between groups'.

**Conclusion**

According to these results the phitofert product for woman can utilize in medicating of hyperprolactinaemic women. And it will be beneficial if they undergoes to IVF[55-56].
References


int Lepidium identification of prolactin. Methods in Lepidium peruvianum oral, anatomical, characterisation of (CJ, 54)


